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CARCINOMAS OF THE SMALL INTESTINE
IN SHEEP

A Thesis presented in partial fulfilment of the requirements
for the Degree of Doctor of Philosophy at Massey University.

Bruce Henry Simpson

1971

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I N T R O D U C T I O N

Carcinomas of the small intestine were long considered uncommon neoplasms in all species. Over the last decade there has been a growing awareness that, contrary to this belief, small intestinal carcinomas are common in sheep, at least in New Zealand. Not only is this high incidence of an otherwise rare neoplasm scientifically intriguing, but it is also of potential economic importance both to individual farmers and to meat slaughtering firms. In addition, the possibility of the existence, in the rural environment, of an unrecognised carcinogen to which man also might be susceptible adds to the importance of this disease. These factors stimulated the initiation of the present investigation into the pathology, prevalence, epidemiology and aetiology of this neoplasm, results of which form the basis of this thesis. Included also are reviews of the pertinent literature concerning small intestinal carcinomas of other species, including man, and of neoplasia of sheep in general. The results of preliminary experiments to investigate the effect of known carcinogens on sheep are also reported.

CHAPTER I

REVIEW OF LITERATURE

Neoplasia in sheep has generally been regarded as infrequent. Similarly, carcinomas of the small intestine are infrequent neoplasms in man and most animals. Despite these two observations, over recent years small intestinal carcinomas have been reported to be common in sheep in New Zealand. In this chapter the available literature on both neoplasia in sheep and carcinomas of the small intestine in all species is reviewed.

NEOPLASIA IN SHEEP

The only informative data on prevalence of neoplasms in sheep have been collected at slaughtering establishments. In the United States of America (Steiner and Bengston, 1951; Moulton, 1963; Brandly and Migaki, 1963) and Great Britain (Anderson, Sandison and Jarrett, 1969) the prevalence of tumours in slaughtered sheep has usually been 17 to 24 per million (0.0017 to 0.0024%). In contrast, Norval (1968) found neoplasms in 0.030% of sheep slaughtered at Edinburgh and Misdorp (1967) diagnosed tumours in 0.027% of sheep slaughtered at Amsterdam, Webster (1966, 1967) recorded a still higher prevalence in sheep slaughtered at a Meat Export Works in New Zealand. In 1965, he found tumours in 0.95% of ewes slaughtered over a 90 day period. By more careful inspection techniques, this figure was increased to 1.4% in ewes slaughtered over a four month period in the following year. Of all

T A B L E IThe Prevalence of Tumours in Slaughtered Animals

Reference and location	Prevalence of Tumours (% of Animals Examined)				
	Sheep	Cattle	Calves	Pigs	Horses
Steiner and Bengston (1951) (U.S.A.)	0.002	0.063	0.003	0.003	0.022
Brandly and Migaki (1963) (U.S.A.)	0.0017	0.2235		0.0036	
Moulton (1963) (U.S.A.)	0.002	0.04		0.003	
Webster (1966) (New Zealand)	0.11				
Misdorp (1967) (Netherlands)	0.027	0.060	0.006	0.004	0.140
Anderson, Sandison and Jarrett (Britain)	(1969) 0.0024	0.023		0.0036	

T A B L E I IMiscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No. of Cases	Reference
Mouth	Gingival fibroma	1	Lombard and Gaubert (1959)
	Squamous cell carcinoma of gum	1	Cotchin (1960)
Oesophagus	Papilloma	1	Anderson <u>et al.</u> (1969)
	Carcinoma	1	Montfallet
Rumen	Leiomyosarcoma	1	Sastry and Tweihaus (1965)
Omasum	Acanthoma	1	Jackson (1936)
	Squamous cell carcinoma	1	Hanko (1947)
Small intestine	Adenomatoid hyperplasia of Brunner's glands	1	Jackson (1936)
Large intestine	Adenocarcinoma	1	Head (1953)
	Cystadenoma of rectum	2	Nobel, Neumann and Klopfer (1970)
Pancreas	Carcinoma	1	Jackson (1936)

Table II Contd.

Miscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No. of Cases	Reference
Peritoneum	Mesothelioma	1	Plummer (1956)
	Carcinoma taken to be of peritoneal origin	1	Saini (1941)
Digestive Organs (Unspecified)	Malignant fibromas or sarcomas	2	Brandly and Migaki (1963)
Nose and Nasal cavity	Squamous cell carcinoma of nostril	1	Sastry (1959)
Lung	Carcinoma	1	Schutz (1880)
		1	Eber (1891)
		1	Besnoit (1895)
		1	Sticker (1902)
		1	Jackson (1936)
		2	Brandly and Migaki (1963)
	Multiple cystadenoma	1	Feldman (1931)
	Visceral mesothelioma	1	Feldman (1931)
	Rhabdomyoma	1	Day (1922)
Mammary Gland	Fibrosarcoma	1	Plummer (1956)
Vulva	Squamous cell carcinoma	1	Monlux, Anderson and Davis (1956)
Uterus	Leiomyoma	2	Feldman (1931)
	Smooth muscle tumour	1	Anderson and Sandison (1969a)

Table II Contd

Miscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No.of Cases	Reference
	Fibroma	1	Anderson and Sandison (1969b)
	Chondroma	1	Sastry (1959)
	Adenocarcinoma	1	Terlecki and Watson (1967)
Ovary	Granulosa cell tumour	1	Casarosa(1950a)
Female Repro- ductive system (Unspecified)	Leiomyoma	1	Brandly and Migaki (1963)
Testis	Intratubular seminomas	5	Jensen and Flint (1963)
	Seminomas	2	Shortridge and Cordes (1969)
	Sertoli cell tumour	1	Shortridge(1962)
Kidney	Adenoma	1	Flir (1952-3)
	Carcinoma	4	Sandison and Anderson(1968b)
	Embryonal nephroma	2	Jackson(1936)
		1	Pamukcu(1956)
		1	Brandly and Migaki (1963)

Table II Contd

Miscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No. of Cases	Reference
Skin	Haemangioendothelioma	1	Harcourt and Spice (1968)
		1	Feldman(1933)
		3	Flir(1952/3)
		1	Webster (1967)
		1	Flir (1952/3)
	Melanoma	1	Lund (1923)
		1	Baxter (1960)
		1	Sastry and Tweihaus(1965)
		1	Anderson <u>et al.</u> (1969)
	Fibroma	1	Jackson (1936)
		1	Head (1953)
		1	Brandly and Migaki (1963)
		2	Cotchin (1960)
	Fibropapilloma	5	Head (1953)
	Papilloma	1	Cotchin(1960)
		1	James, Rao and Sastry (1967)
	Cornu cutaneum	1	Jackson(1936)
	Adenocarcinoma (Cystic type)	1	Feldman(1931)

Table II Contd.

Miscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No. of Cases	Reference
Eye	Capillary haemangioma (congenital)	1	Davis (1935)
	Melanoma	1	Cotchin (1960)
	Squamous cell carcinoma	1	Sastry and Tweihaus(1965)
		2 4	Feldman (1932) Davis and Shorten(1952)
Lacrimal	Adenocarcinoma of accessory lacrimal glands	1	Micheletto(1968)
	Neurofibrosarcoma	1	James <u>et al.</u> (1967)
	Tumours (unspecified)	2	Davis, Leeper and Shelton (1933)
Nervous system	Lipoma of meninges (congenital)	1	Jackson (1936)
	Benign neurofibroma or Neurolemmoma	1	Brandly and Migaki (1963)
	Meningioma	1	Pivnik(1968)
Anterior pituitary	Adenoma	1	Casarosa (1950 b)
Thyroid	Carcinoma	1	Feldman(1931)
		3	Jackson(1936)
		1	Sandison and Anderson(1968 a)

Table II Contd

Miscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No.of Cases	Reference
	Adenoma	1	Nobel and Neumann(1960)
	(after radioiodine administration)		Marks and Bustad(1963)
	Tumour (unspecified)	1	Davis <u>et al.</u> (1933)
Adrenal	Carcinoma of cortex	9	Feldman (1931)
		1	Sandison and Anderson(1968a)
	Adenoma of cortex	1	Feldman(1931)
		1	Cotchin(1960)
	Benign adrenal medullary tumour	1	Brandly and Migaki (1963)
	Pheochromocytoma	1	Sandison and Anderson(1968a)
	Carcinoma (Unspecified)	8	Davis <u>et al.</u> (1933)
	Adenoma (Unspecified)	1	Davis <u>et al.</u> (1933)
Bone	Osteoma	1	Jackson(1936)
		1	Cotchin(1960)
	Osteosarcoma	1	Feldman(1931)
		2	Webster(1967)

Table II Contd

Miscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No.of Cases	Reference
Connective Tissue	Fibrosarcoma	1	Jackson(1936)
		1	Feldman(1932)
	Fibroblastic tumours	3	Anderson and Sandison(1969b)
	Fibrolipoma	1	Jackson(1936)
	Lipoma	1	Anderson and Sandison(1969b)
	Mixed cell sarcomas	1	Jackson(1936)
	Myxoma	1	Jackson(1936)
	Rhabdomyoma	1	Feldman(1932)
	Rhabdomyosarcoma of carcass	1	Harcourt and Spice (1968)
	Rhabdomyosarcoma	1	Collet, Collin and Flachet (1952/53)
	Haemangioendothelioma	2	Anderson and Sandison(1969b)
	Haemangioma	1	Webster (1967)
	Lymphangioendothelioma	1	Anderson and Sandison(1969b)
Thymus	Thymoma	1	Santos and Costa Durao(1965)
		2	Brandly and Migaki(1963)
		1	Anderson <u>et al.</u> (1969)

Table II Contd.

Miscellaneous Neoplasms of Sheep Diagnosed
by Various Authors

Anatomical Region	Tumour Type	No. of Cases	Reference
	Carcinoma (Probably of Hassells corpuscles)	1	Feldman(1931)
	Thymus tumour	1	Davis <u>et al.</u> (1933)
	Malignant thymic tumour	1	Nyka(1929)
Heart	Sarcoma	1	Cotchin(1960)
	Fibrosarcoma	1	Misdorp(1962)
	Osteosarcoma	1	Misdorp(1962)
Unknown Origin	Encephaloid tumour of chest	1	Crisp(1867)
	Carcinoma	1	Savage and Isa (1955)
	Adenoma	7	Nobel <u>et al.</u> (1970)

sheep, including lambs, slaughtered over that period, 0.25% were reported as having neoplasms, but of these, 59% were a splenic lesion of uncertain nature. Jolly (1968) subsequently described these particular lesions as nodular hyperplastic changes rather than neoplastic. It is therefore reasonable to omit these splenic lesions from Webster's data, leaving a prevalence of 0.58% of neoplasms in ewes and 0.11% in all sheep in Webster's latter survey.

In comparison with the prevalence in sheep, reports of investigations at slaughtering establishments have usually revealed considerably more tumours in slaughtered cattle, but approximately the same number in pigs (Table I). The two reports from slaughtered horses indicate a considerably higher prevalence in that species. Webster (1966) did not state how many cattle were inspected to find the sixteen neoplasms he reported. He did, however, in contrast to other authors, state that the prevalence in cattle was much lower than the tumour prevalence in sheep.

Despite the relative infrequency of diagnoses of ovine neoplasms in most countries, a wide range of types of neoplasms has been reported, affecting most organs and tissue types (Table II). Apart from these, a number of characteristic neoplasms occur with regularity in certain circumstances. These, omitted from Table II, are discussed in more detail below.

Sheep Pulmonary Adenomatosis (Jagziekte)

One of the most interesting of ovine neoplasms is pulmonary adenomatosis (jagziekte), a disease recognised at least since the turn of the century (Robertson, 1904), and since reported in Peru, Chile, Iceland, United Kingdom, most of Europe, Southern Russia, Israel, India, Kenya, South West Africa and South Africa (Tustin, 1969).

In spite of this, considerable confusion exists over its differential diagnosis through failure to recognise the adenomatous lesion as being neoplastic, and to differentiate it from pneumonic lesions characterised by alveolar epithelialisation. Robertson, in fact, described jagziekte as a chronic catarrhal pneumonia. Cowdry and Marsh (1927) considered it to be the same condition as Montana progressive pneumonia, although Cowdry (1925 a, b) had previously commented on the proliferative nature of the disease, and in their comparison between jagziekte and Montana progressive pneumonia, Cowdry and Marsh recognised that alveolar epithelial proliferations were more marked in the former. Marsh (1955) later recognised the two conditions as separate entities, with progressive pneumonia more closely resembling Maedi, a proliferative pneumonia of sheep described from Iceland (Sigurdsson, 1954). Sheep pulmonary adenomatosis was recognised by Dungal, Gislason and Taylor (1938) as being primarily an adenomatous condition accompanied by inflammatory changes. The frankly neoplastic nature of this lesion was confirmed by the noting of secondary lesions in thoracic lymph nodes by Cuba-Caparo, de la Vega and Copaira (1961), Stamp and Nisbet (1963), Wandera (1967), Nobel et al. (1969) and Cvjetanovic, Forsek and Nevjestic (1970). Metastases elsewhere than the thoracic lymph nodes have been reported by Enchev (1963), Nobel et al. (1968, 1969) and Tustin (1969). Enchev reported metastatic lesions in the liver, spleen, kidney and myocardium, while Nobel et al. reported metastatic lesions in skeletal muscle, kidneys, the "pelvic cavity" and in the peritoneal cavity. Tustin observed two sheep in which lesions had spread to involve the pleura. On the basis of the secondary lesions observed, Nobel et al. (1969) and Tustin (1969) suggested this disease should be called "carcinoma" of the lung.

The transmissible nature of jagziekte was suspected by Robertson (1904) and Cowdry (1925 b). An epizootic of this disease, affecting up to 50% of sheep in some Icelandic flocks, was investigated by Dungal et al. (1938), who traced the introduction of the disease into Iceland to a ram imported from Germany. Experiments by Dungal (1946) confirmed that the disease was contagious and suggested that a pneumotropic virus was responsible.

Experimental transmission of sheep pulmonary adenomatosis using cell free and bacteria free preparations has now been achieved in a number of laboratories (Sigurdsson, 1958; Enchev, 1966; Cuba-Caparo, de la Vega and Durand 1967; Wandera 1968), suggesting a P.P.L.O. or viral aetiological agent. Naturally occurring lesions are generally accompanied by mesenchymal proliferation and leukocytic infiltration of alveolar walls. Experimental transmission using bacteria free preparations has, however, confirmed that pulmonary adenomatosis is primarily characterised by adenomatous proliferation of alveolar epithelium. Mycoplasmas have been isolated from lesions of the naturally occurring disease (Mackay, Nisbet and Foggie, 1963; Deiana and Cerotto, 1967), although their aetiological significance is unknown. In studies in which tissue cultures were seeded with material from characteristic lesions, either proliferative or cytopathic effects were produced (Mackay, 1969). Examination of preparations from these tissue cultures with an electron microscope revealed particles morphologically similar to herpes viruses (Smith and Mackay, 1969). The aetiological significance of this virus has still to be demonstrated.

Neoplasia of the Olfactory mucosa

Multiple cases of tumours of the olfactory mucosa, variously classified as adenopapillomas and adenocarcinomas have occurred in individual sheep flocks in Europe (Nierberle, 1939 ; Cohrs, 1953 ; Camy, 1955 ; Gussman, 1962 ; Rubaj and Wolosyn, 1967), the United States of America (Young, Lovelace, Hawkins and Catlin, 1961 ; Duncan, Tyler, Van Der Maaten and Anderson, 1967), and South Africa (McConnell, van Rensburg and van Wyk, 1970). These neoplasms arose in the olfactory and ethmoid regions of the nasal cavity and appeared as red brown thickenings of the mucosa that grew to occlude the nasal passage producing clinical signs of dyspnoea (Goldberg, 1920; Camy, 1955; Young et al., 1961). Histologically the lesions varied from diffuse carcinomas (Goldberg, 1920) to papillary carcinomas with signs of squamous metaplasia (Cohrs, 1953; Young et al., 1961; Gussman, 1962). No metastases have been reported.

Genetic predisposition does not appear to account for the prevalence of this disease on individual farms, as Young et al. found tumours affecting sheep of different breeds on the one property. Whereas Cohrs (1953) was able to transmit this tumour using a cell free filtrate, similar attempts by Duncan et al. (1967) were unsuccessful.

Whether or not all these neoplasms were a single pathological entity with a similar aetiology has not been determined.

Lewis, Markson and Allcroft (1967) found neoplasms of the nasal cavity in two out of five sheep fed for up to five years on ground nut meal containing aflatoxin. One of these neoplasms was a chondroma and the other was described as a mucous adenoma with osteomatous differentiation in the stroma.

Squamous Cell Carcinomas of the Exposed Skin

While squamous cell carcinomas on the ears and other areas of exposed skin of sheep are well known (Jubb and Kennedy, 1963), there are only a few reports in the literature from places other than Australia (Beatti, 1916; Feldman, 1931; Head, 1953; Anderson et al. 1969; Nobel et al. 1970). In Australia, these neoplasms are common on the tips of ears arising from areas which have undergone chronic inflammatory changes (Dodd, 1923). Up to 8% of ewes were affected in flocks examined by Carter (1958) and Lloyd (1961), with lesions affecting the ears, muzzle and perineum. Metastatic lesions have been located in lymph nodes and, more rarely, in the lungs (Dodd, 1923; Lloyd, 1961).

Beatti (1916) suggested penetration of the ears by thorns might be responsible for this neoplasm as seen in Argentina, while Dodd considered chronic granulating ear mark wounds might be the site of origin. The ultra-violet portion of sunlight is now well recognised as being carcinogenic to skin (Blum, 1955; Hueper, 1957), and Lloyd considered sunlight was the major aetiological factor in squamous cell carcinoma of the exposed portions of the skin of sheep. He suggested that a preceding period of photosensitivity was responsible for the especially high incidence in flocks examined by Carter and himself.

Squamous Cell Carcinomas of the Wool Bearing Skin

Squamous cell carcinomas of the wool bearing areas of the skin of sheep have rarely been reported. Feldman (1931) recorded two such neoplasms originating in the region of the scapula, and Sastry (1959) recorded one case arising on the neck. In contrast, Carne, Lloyd and Carter (1963) found squamous cell carcinomas originating in epidermal

TABLE III

The numbers of Neoplasms of Different Types
Diagnosed by Various Authors

Reference	Primary Liver Tumours	Lymphosarcomas	Cartilagenous Tumours	Other Tumours
Sticker(1902)	5	1	0	1
Feldman(1931)	5	4	2	29
Jackson(1936)	3	4	0	21
Monlux <u>et al.</u> (1956)	8	20	3	35
Sastry (1959)	1	0	1	2
Cotchin (1960)	12	10	2	14
Lombard,Guilbard and Rimbert (1960)	0	1	1	0
Brandly and Migaki(1963)	8	11	3	12
Moulton(1963)	?	77	?	163
Sastry and Tweihaus (1965)	0	3	2	5
Webster(1966)	27	23	2	68
Webster(1967)	34	7	2	342
Misdorp(1967)	6	16	?	10
Anderson, <u>et al.</u> (1969)	33	44	3	27

T A B L E I VHepatic Tumours Diagnosed by Various Authors

Reference	Hepatocellular carcinomas and	Cholangio- cellular carcinomas or Bile Duct adenomas	Others	Unspecified
Johne (1879)	1			
Besnoit(1895)	1			
Sticker(1902)				5
Hodgson(1903)				1
Frenkel(1927)				6
Feldman(1931)	2	2	1 Carcinoma (uncertain cell type)	
Jackson(1936)	2			
Plummer(1956)		3		
Pamukcu(1956)			1 angiosarcoma	
Monlux et.al. (1956)				8
Sastry (1959)	1			
Cotchin(1960)	8	3	1 uncertain Bile Duct Carcinoma	
Pierotti(1963)	2		5 adenocarcinomas	
Brandly and Migaki(1963)	5	3		
Rao, Christopher and Sastry (1964)		1		
Manktelow(1965)			13 Hepato- blastomas	
Webster (1966)	20	7		
James et al. (1967)			1 Cavernous haemangioma	

Table IV Contd

Hepatic Tumours Diagnosed by Various Authors

Reference	Hepatocellular carcinomas and Hepatomas	Cholangio- cellular carcinomas or Bile Duct adenomas	Other	Unspecified
Misdorp(1967)	5		1 Carcino- sarcoma	
Webster(1967)			2 Hepato- blastomas	32 (liver carcinomas)
Anderson and Sandison (1968)	23	9	1 Bilary cystadenoma	
Watt (1970)			1 Hepatochol- angioma	

implantation cysts in the wool bearing skin of sheep. The sheep in three related inbred Merino flocks were genetically susceptible to the formation of cutaneous cysts following the penetration of the skin by grass seeds. It was from the walls of these cysts that the neoplasms originated.

Experimentally, the application of dimethylbenzanthracene to the skin resulted in papilloma formation, more commonly in the cyst-susceptible sheep than in other sheep, and the injection of dimethylbenzanthracene into the lumen of cysts stimulated the formation of squamous cell carcinomas (Lloyd, 1966). An electron microscopic study of these tumours was described by Borland and Webber (1966).

Hepatic Neoplasms

Primary neoplasia of the liver is not uncommon in sheep compared with neoplasia in other organs (Table III). Steiner and Bengston (1951) considered hepatocellular carcinomas were the most common malignant neoplasm found in sheep, while Norval (1967) believed the liver was second to the small intestine as the most common site in which tumours were found in slaughtered sheep.

Tumours of the liver have usually been classified as arising from either hepatocytes or bile duct epithelium, but there is variation in the terminology used by various authors (Table IV). Webster (1967) failed to classify tumours of the liver beyond calling them liver carcinomas. However, in his earlier survey (Webster, 1966) hepatocellular tumours predominated and this is in accord with other series of liver tumours.

Haemopoiesis in liver cell tumours of sheep has been reported by a number of authors (Frenkel, 1927; Drieux and Ninard, 1942; Nunes Pestica, Paz Ferreira and Carvalho Varela, 1961; Manktelow, 1965; Anderson and Sandison, 1968). On the basis of this finding, and other pathological considerations, Frenkel (1927) suggested these lesions might best be called hepatoblastomas, and this classification was supported by Nunes Pestica et al. (1961). Further evidence that these lesions were of embryonic origin was the finding of similar lesions in newborn lambs (Manktelow, 1965). Webster called liver cell tumours in lambs hepatoblastomas, but those in older sheep he classified as hepatocellular carcinomas. Anderson and Sandison (1968), who also found haemopoiesis in liver tumours from lambs, preferred to classify them only as "liver cell tumours".

Though it has been inferred that liver cell tumours of sheep are commonly malignant (Steiner and Bengston, 1951; Nunes Pestica et al., 1961), this is not supported by most authors. Metastatic lesions were located in approximately one-fifth of the cases examined by Brandly and Migaki (1963), Webster (1966) and Anderson and Sandison (1968). These last authors located metastases in two of the twenty-one cases of liver cell tumours examined. In the absence of metastases they considered a further three lesions malignant on histological criteria.

Lymphosarcomas

Lymphosarcomas are one of the most commonly recorded tumours of sheep (Table III). Anderson and Jarrett (1968) considered most cases were multicentric, involving numerous lymph nodes. Frequently

internal organs such as kidney, spleen, liver, lung and skeletal muscle were also involved.

Isolated reports of diagnoses of lymphosarcomas in sheep have come from Jowett (1906), Feldman (1926), Head (1953), Lombard and Witte (1959), Hilgenfeld and Krieg (1965), Nobel et al. (1967) and Dukic, Stamatovic and Durickovic (1969).

An extensive volume of literature is available on lymphosarcoma of cattle in which species an enzootic form of possible viral aetiology is present (Bendixin, 1965; Dutcher, Larkin, Tumilowicz, Nazarian, Eusebio, Stock, Guest and Marshak, 1967). Similarly, there are indications of a transmissible aetiological agent in feline lymphosarcoma (Schneider, Frye, Taylor and Dorn, 1967; Theilen, 1968). Despite this, and the relatively common occurrence of lymphosarcoma in sheep, no experimental work and few epidemiological observations on the ovine disease have been reported.

Enke, Jungnitz and Robger (1961) reported the existence of a flock of sheep with a high incidence of malignant lymphoma, similar to the situation in certain herds of cattle. Observations on a flock of sheep vaccinated two years previously with bovine blood from a leukosis herd, suggested that transmission of the cattle leukosis agent might have induced a high incidence of leukosis in that sheep flock (Enke, 1964). An attempt by Farny (1935) to induce lymphosarcomas in sheep by inoculation of three animals with both centrifuged and filtered sera from a leukotic cow resulted in a "lymphocytosis" but no discrete neoplasms. Wittman and Urbaneck (1969), however, were able to induce "leukosis" in sheep by inoculating them with heparinised blood from leukotic cattle during the first 24 hours of life. There are no reports of successful transmission of lymphosarcoma from sheep to sheep.

Despite this, evidence for a viral aetiology for this disease in other species and the observations that bovine leukaemia may be transmitted to young lambs suggest the possibility of an infective cause for the ovine disease.

Tumours of Cartilage

Although Steiner and Bengston (1951) claimed cartilagenous tumours, chiefly of the scapular and thoracic cage, were relatively common in sheep, they form only a small proportion of most series of ovine neoplasms (Table III). Most have been diagnosed as chondromas. In contrast, Sullivan (1960) examined histological sections from twenty-two lesions in sheep and considered only two could be classed as benign. Records of thirty-two cartilagenous neoplasms of sheep were available to Sullivan, and of these, eleven arose on the ribs, eight on the sternum, four on the scapula, four on various articulations and one on a tuber coxae. Two lesions were located in the heart and two cases were recorded as multiple metastatic nodules only. Metastases were reported in the lungs (10 cases), liver (5), diaphragm (5), heart (1), intestine (1), and kidney (1). Hock (1922) diagnosed a cartilagenous neoplasm of the proximal epiphysis of the femur in a sheep with secondary lesions in the lung.

Carcinoma of the Bladder

Harbutt and Leaver (1969) found carcinomatous lesions of the urinary bladder associated with haematuria in sheep which had access

T A B L E V

The Number of Small Intestinal Carcinomas Diagnosed
in Series of Ovine Tumours

Reference	Sheep Examined	Tumours Examined	Small Intestinal Carcinoma Diagnosed
Feldman (1931)		40	Nil
McFadyean (1931)			1*
Davis, <u>et al</u> , (1933)		34	Nil
Jackson (1936)		39	1
Monlux, <u>et al</u>	1.11×10^6	66	1*
Cotchin (1960)		38	1*
Dodd (1960)			51**
(1964)			
Brandly and Migaki (1963)		34	Nil
Cook (1964)	88		6***
Sastry and Tweihaus (1965)		10	2*
McDonald and Leaver (1965)			5**
Webster (1966)	30,450 (ewes)	62	37
Webster (1967)	363,500	385	339
Misdorp (1967)		32	Nil
Norval (1968)	537,946 (1966 and 1967 Figs)	164	96
Anderson, <u>et al</u> (1969)	4.5×10^6	107	1

* The primary site of these lesions was not fully stated and they could have arisen in the large intestine.

** These reports are restricted to carcinoma of the small intestine.

*** Cook reported these lesions as "neoplasia of the intestine" only.

to bracken fern for at least 18 months. On the basis of the proven carcinogenicity of bracken fern (Chapter V), and the similarity of these lesions to those produced by feeding bracken fern to cattle, it was suggested that these tumours resulted from bracken ingestion.

Carcinomas of the Small Intestine

Prior to 1960, carcinomas of the intestine of sheep had been reported only occasionally and some of these early reports failed to state the portion of the intestine from which the tumours originated (Table V). Dodd (1960, 1964) reported a total of fifty-one cases of carcinoma of the ovine small intestine in material passing through a New Zealand diagnostic laboratory over a period of seven years. Six of the eighty-eight "wasting" sheep examined by Cook (1964) in New Zealand were affected by "neoplasia of the intestine". The common occurrence of small intestinal carcinomas in ewes in New Zealand was confirmed by Webster (1966, 1967).

In Australia, McDonald and Leaver (1965) diagnosed carcinoma of the small intestine as the cause of death of five sheep on one property. They considered this disease was causing the death of 0.4% of all sheep and 2% of sheep over five years of age on that property, but could not locate the disease on surrounding farms.

The only locality outside New Zealand in which relatively large numbers of carcinomas of the small intestine are known to occur is Scotland. Norval (1968) found between 0.015 and 0.048% of sheep slaughtered at Edinburgh Abattoir affected each year from 1962 to 1967. This contrasts markedly with the situation in Great Britain

as a whole, as reported by Anderson et al. (1969), who found one carcinoma of the small intestine in 4.5×10^6 sheep examined.

Webster (1967) diagnosed carcinomas of the small intestine in 0.52% of slaughtered ewes, and in approximately 0.02% of hoggets and wethers. No cases were noted in over 200,000 slaughtered lambs. Norval stated that his cases of small intestinal carcinoma came from aged ewes, and, as these constituted 15 to 20% of sheep killed, the prevalence in ewes would be approximately 0.2%.

Husbandry practices generally preclude the possibility of examining large numbers of slaughtered rams of comparable age to the slaughtered ewes, and hence no comparison of prevalence in different sexes is possible.

The view that ovine neoplasms are of generally low prevalence is supported by the literature reviewed but with several exceptions. The most notable exceptions are the common occurrence of small intestinal carcinoma in slaughtered ewes in New Zealand and Scotland, and the comparatively high prevalence of total tumours in sheep slaughtered in these two localities and Amsterdam. Other exceptions include the endemic and sometimes epidemic occurrence of pulmonary adenomatosis, tumours of the olfactory mucosa, squamous cell carcinoma of the exposed skin and squamous cell carcinoma in cyst-bearing, genetically susceptible sheep.

T A B L E V I

The Numbers of Small Intestinal Carcinomas Diagnosed
in Series of Bovine Tumours Examined by Various Authors

Reference	Animals Examined	Tumours Examined	Small Intestinal Carcinoma Diagnosed
Chandresekharan-Nair and Sastry (1954)		2003	Nil
Plummer (1956)		447	10*
Brandly and Migaki(1963)			5*
Sastry and Tweihaus(1964)		202	Nil
Webster (1966)	7×10^4	16	1*
Misdorp (1967)	24.7×10^4	208	12
Anderson, <u>et al.</u> (1969)	130×10^4	302	1*

* The primary site of these tumours was not positively identified.

COMPARATIVE ASPECTS OF THE OCCURRENCE OF CARCINOMA OF THE SMALL INTESTINE

As already noted, sheep, at least in New Zealand, and to a lesser degree in Scotland, are notable for a relatively high prevalence of small intestinal carcinomas compared with the prevalence of other neoplasms. Reports of carcinomas of the small intestine in all other animal species have been infrequent and generally indicate that they are an uncommon type of neoplasm. Many authors have failed to differentiate between carcinomas of the small and large intestine, and few have defined the population size or type from which they originated. Veterinary pathology texts consider that intestinal carcinomas are rare in all domestic species (Runnells, Monlux and Monlux, 1960; Smith and Jones, 1963; Jubb and Kennedy, 1963; Nierberle and Cohrs, 1967). Moulton (1961) considered carcinomas of the intestine were most frequent in the dog, followed by the horse, cat, ox and sheep. Nierberle and Cohrs (1967), however, stated that intestinal carcinomas were most common in horses and fowls.

Standard medical texts (Boyd, 1949; Robins, 1962; Morson, 1966; Horn, 1966; Gould, Hinerman, Batsakis and Beamer, 1964; Willis, 1967) consider that carcinoma of the small intestine is a rare neoplasm in man.

Cattle

Table VI contains reports of several series of bovine neoplasms examined in various laboratories, together with the number of small intestinal carcinomas diagnosed. The reports of Brandly and Migaki (1963), Webster (1966), Misdörp (1967) and Anderson et al. (1969)

T A B L E V I I

The Numbers of Small Intestinal Carcinomas Diagnosed
in Series of Canine Neoplasms

Reference		Tumours Examined	Small Intestinal Carcinomas Diagnosed
Uberreiter	(1955)	3040	Nil
Plummer	(1956)	83	1*
Cotchin	(1959)	4187	3
De Kock	(1962)	72	1*
Howard and Nielsen	(1965)	1053	Nil
Stunzi	(1967b)	212	Nil
Murray	(1968)	189	Nil
Mugera	(1968)	559	2*
Hoorens and Thooren	(1968)	925	Nil

* The primary site of these lesions was not positively identified. They could have arisen in the large intestine.

indicate a prevalence of intestinal carcinoma in slaughtered cattle ranging from approximately 0.0001 to 0.005%. This prevalence range is considerably below that recorded from sheep in New Zealand and Scotland, but is similar to data from sheep in the rest of Britain or in the United States of America (Anderson et al. 1969; Monlux et al. 1956).

Schlegel (1925) reported the diagnosis of carcinomas of the small intestine in three aged cows and Kajagoschtin, Zeinert (1931), and Dzuvic (1969) each reported one case.

Dogs

Table VII summarises reports of series of canine neoplasms, and the numbers of carcinomas located in the small intestine. These reports indicate that carcinomas of the small intestine form a smaller proportion of canine neoplasms than is the case in either sheep or cattle.

Isolated cases of small intestinal carcinomas in dogs have also been diagnosed by Petit (1905), Joest (1919), Steckentorn (1933), Rubarth (1934), Taylor and Kater (1954), while Wuller (1954) and Case, Helper, Knecht and Small (1968) each recorded two such lesions.

Krook (1956) reported a further eight small intestinal carcinomas from dogs, but gave no information on the population size from which these cases were drawn. He found that dogs over 6 years old were predominantly affected and that no breed predisposition was evident. Krook (1954) indicated that carcinomas of both the small and large intestine accounted for 2.05% of canine carcinomas at the Royal Veterinary College, Stockholm.

T A B L E VIII

The Number of Small Intestinal Carcinomas
Diagnosed in Series of Feline Neoplasms

Reference	Tumours Examined	Small Intestinal Carcinomas diagnosed
Cotchin (1959)	571	10
Head (1959)	571	8 *
Sastry and Tweihaus (1965)	14	4 *
Brodey (1966)	46 **	3
Schmidt and Langham (1967)	256	1 *
Stunzi (1967a)	86 **	Nil
Whitehead (1967)	165	2

* The site of origin of these tumours was not defined.

They could have arisen in the large intestine.

** This article is restricted to tumours of the alimentary tract only.

Cats

Table VIII summarises reports of series of feline neoplasms and the numbers of carcinomas located in the small intestine. These reports indicate that carcinomas of the small intestine form a similar proportion of total feline neoplasms to those in cattle and a larger proportion than in dogs.

Petit (1902), Joest (no date), Antoine and Brouwers (1941), Taylor and Kater (1954) and Theran and Thornton (1968) have each reported single cases of carcinoma of the small intestine in cats.

Few authors have stated the age of cats with small intestinal carcinomas, however that reported by Taylor and Kater (1954) was 8 years old, by Theran and Thornton (1968) 14 years, and one "probable carcinoma" reported by Cotchin (1959) was in a 2 year old animal.

Brodey (1966) noted no significant sex difference in the occurrence of gastro-intestinal neoplasia in cats.

Horses

Samso and Catanei (1952), in a review of Continental European literature on intestinal neoplasia, found twelve references to twenty-three tumours of the small intestine of horses, none of which were referred to as carcinomas. Most were sarcomas of varying types, but others were mentioned merely as "cancer". Also quoted was a survey by Cesari who diagnosed intestinal carcinomas in 0.04% of 40,000 abattoir-killed horses but did not state the portion of intestine from which they arose.

Rubarth (1934) and Antoine and Brouwers (1941) each recorded one case of equine small intestinal carcinoma.

Pigs

Intestinal carcinomas have not been reported in pigs.

Goats

One carcinoma of the intestine of a goat was diagnosed by Brandly and Migaki (1963), but they failed to define its site of origin.

Non-domesticated Mammals

Isolated cases of carcinoma of the small intestine in animals in zoological gardens have been reported in Felis leo (lion), Procyon lotor (raccoon), Dasyurus maculatus (Australian tiger cat) (Ratcliffe, 1933), Panthera tigris (tiger) and Mephitis mephitis (skunk) (Lombard and Witte, 1959).

Laboratory Animals

"Spontaneous", as opposed to experimentally induced, carcinomas of the small intestine have rarely been diagnosed in laboratory rats and mice. Rowlatt (1967), in an extensive review, noted references to only one such lesion in a rat and three in mice. Fortner (1957, 1958) reported the occurrence of small intestinal carcinomas, usually in association with large intestinal lesions, in a number of hamsters.

A condition described by Jonas, Torita and Wyand (1965) as "enzootic intestinal adenocarcinoma" in hamsters did not give rise to metastases and was considered by Dodd (1968) to be an adenomatous

hyperplasia, similar to a condition he described in pigs.

Birds

Most lesions previously classified as carcinomas of the intestine in birds were considered by Jackson (1936) to have been metastatic lesions from tumours of the reproductive tract. Jackson considered earlier suggestions that carcinomas of the intestine were common in birds were wrong.

Small intestinal carcinomas have been diagnosed in fowls (Petit and Germain, 1909; Jackson, 1936), an ostrich (Joest and Ernesti, 1916), a mallard duck (Anus platyrhynchos) (Ratcliffe, 1933), a duck (Fox, 1927) and a bittern (Botaurus lentiginosis) (Fox, 1923). The site of origin of an intestinal carcinoma diagnosed by Lombard and Witte (1959) in a Canadian goose (Branta canadensis) was not defined.

Neither Arnall (1961) nor Blackmore (1966) located any carcinomas of the intestine in extensive collections of avian neoplasms.

Humans

The first description of carcinoma of the duodenum is believed to be that of Hamberger (1746). Sorlin (1824) was the first to describe a carcinoma of the jejunum. Since those times, a considerable volume of literature has accumulated on this subject, consisting principally of reports of only a few cases.

T A B L E I X

Proportion of Malignant Tumours of the Small
Intestine in Man which are Carcinomas

	Reference	Total Malignant Tumours	Carcinomas
Tuttle	(1905)	59	24
Raiford	(1932)	38	16
Fraser	(1945)	21	12
Doub	(1947)	47	34
Roddy	(1957)	13	8
Dockerty	(1958)	167	63
Darling and Welch	(1959)	110	38
Arlich, Blank and Fomon	(1960)	15	5
Krouse, Everly and Babcock	(1961)	12	4
Botsford, Crowe and Crocker	(1962)	44	22
Wianko and McKenzie	(1963)	14	2
Strauch	(1964)	47	17
Andersen, Iversen and Østberg	(1965)	4	3
Totals		591	248

T A B L E XAge and Sex Distribution of Human Patients with
Carcinoma of the Small Intestine

Reference	Site	Age Range (years)	Average Age (years)	Number of Cases	Sex Distribution	
					Male	Female
Craig (1923)	S.I.*		47.5	36	29	7
Rankin and Mayo (1930)	S.I.	32 - 69	47.5	55	37	18
Ross (1931)	Jejunum & Ileum	49 - 70	58	4	2	2
Raiford(1932)	S.I.	33 - 68	52	16	11	5
Hoffman and Pack (1937)	Duodenum		53	18	6	12
Heggs (1939)	Jejunum	26 - 53	33	3	2	1
Boman (1944)	Jejunum & Ileum	27 - 71	51	7	4	3
Joergenson, Weibel and Keasbey(1953)	Duodenum			37	25	12
Barclay and Kent (1956)	Duodenum	24 - 83	59	8	3	5
Colcock and Adamson(1959)	Jejunum & Ileum	30 - 79	55	34	16	18
Arlich, Blank and Fomon (1960)	S.I.	41 - 74	56	5	1	4
Wianko and MacKenzie(1963)	S.I.	65 - 66	66	2	0	2
Anderson, Iversen and Østberg (1965)	Duodenum	36 - 61	49	3	1	2

* Small Intestine (all portions)

Data collected by National Cancer Registries have been published only in terms of organs from which the tumours arose, omitting histological subdivisions. Such publications, therefore, do not give precise information on the occurrence of carcinomas in the small intestine. Information available from other sources (Table IX), however, indicates that approximately 40% of malignant neoplasms of the small intestine are carcinomas.

Cancer registry data compiled by Doll, Payne and Waterhouse (1966), Segi (1960) and Segi, Kurihara and Matsuyama (1969) show that approximately 0.25% of malignant tumours arose in the small intestine. Calculations from Segi's data indicate an overall annual mortality rate from all malignant tumours of the small intestine of 4.22 per 100,000. Geographical differences in incidence were small, with the exception of Belgium, where the mortality rate was 24 per 100,000 males and 34 per 100,000 females. Over all, there was a slightly higher male mortality than female mortality rate (4.39 versus 4.05 per 100,000; $\chi^2 = 7.6$; $p < 0.01$).

The age and sex distributions of persons diagnosed with carcinoma of the small intestine by a number of authors are shown in Table X. The 3 : 2 male : female ratio in this table supports the contention of a number of authors (Eusterman, Berkman and Swan, 1925; Rankin and Mayo, 1930; Eger, 1933; Mayo and Nettour, 1937; Stewart and Lieber, 1937; Prigden, Mayo and Dockerty, 1950; Strauch, 1964; N.Z. Cancer Data, 1966) that more males than females developed carcinomas of the small intestine. Wood (1967) stated that carcinoma of the duodenum was more common in males, that jejunal cases in males outnumbered those in females 2 : 1, and that, in the ileum, there was no sex difference. No figures were produced in support of this contention.

CHAPTER II

THE PATHOLOGY OF CARCINOMAS ARISING IN THE SMALL INTESTINES OF SHEEP

INTRODUCTION

The term "carcinoma", used in reference to neoplasms of the intestines, is usually reserved for malignant tumours considered to have arisen from epithelial cells lining the crypts and villi of the intestinal mucosa. Neoplasms of the argentaffin cells are referred to as "argentaffin cell carcinomas", "carcinoid tumours" or "carcinoids".

The Pathology of Small Intestinal Carcinomas in Man

Approximately one half of carcinomas of the small intestine in man arise in the duodenum. Of these, more than 50% originate in the region of the papilla of Vater. In the jejunum-ileum, carcinomas tend to be concentrated at either extremity, with more occurring in the jejunum than in the ileum (Jefferson, 1916-1917; Deaver and Ravdin, 1920; Eusterman et al., 1925; Eger, 1933; Hoffman and Pack, 1937; Cameron, 1938; Swan, 1946; Prigden et al., 1950; Wood, 1967).

Wood (1967) states that carcinomas of the intestine may take one of four main forms. The most common in the duodenum are the "polypoid - ulcerative" and "infiltrative" forms, while in the jejunum and ileum, carcinomas are usually of the "annular, stenosing,

constrictive" form. The "diffuse" type of carcinoma is rarely seen in the small intestine. The cells of these tumours are predominantly "cylindrical" and the stroma varies in quantity, but is usually scanty. Occasionally, mucoid adenocarcinomas are seen and, rarely, adenoacanthoma and squamous cell types of carcinoma develop.

Obstruction of the small intestine as a result of tumour growth, with consequent dilatation of the lumen proximally, is usually the main cause of clinical symptoms. Perforation of the intestinal wall may occur (Prigden et al., 1950) but is a relatively rare occurrence (Higgins, Lehman and Morton, 1963).

Metastases in the mesenteric lymph nodes, liver and lungs are common (Rankin and Mayo, 1930; Mayo and Nettour, 1937; Fraser, 1945; Wood, 1967). Secondary lesions may also involve the pancreas (Wood, 1967), the peritoneum and omentum (Boman, 1944; Sullivan and Hasbrouck, 1960), the long bones and the dura mater (Rankin and Mayo, 1930). Transcoelomic spread is reported to the diaphragm, pelvic wall, ovary (Stewart and Lieber, 1937; Thomas and Boothroyd, 1953) and uterus (Weingarten, Parker, Chazen and Jacobson, 1959). Transplantation spread along the lumen of the intestine has not been reported, and, according to Heggs (1939), does not occur.

The Pathology of Small Intestinal Carcinomas in Sheep

A single lesion of carcinoma of the small intestine of a sheep was described as being sausage-shaped and firm (Jackson, 1936). The portion of the lesion protruding into the intestinal lumen was lobulated and ulcerated. Stenosis at the primary lesion resulted in the proximal intestine being dilated. Confluent lesions on the

serosa were also noted. Muscular hypertrophy was present in the vicinity of the primary lesion. Histologically, there was invasion of the lamina propria and the submucosa, with penetration of the internal muscle layer by radial strands of neoplastic tissue. The tumour affected the external muscle layer to a lesser extent. The mitotic index of this lesion was two.

The first description of ovine small intestinal carcinoma based on a series of lesions was that by Dodd (1960), who had examined 25 such lesions. McDonald and Leaver (1965) and Webster (1966) have since added descriptions of lesions encountered by them.

Dodd and Webster each reported primary lesions as occurring in the central and distal thirds of the small intestine, though McDonald and Leaver found tumours originating only 3-4 metres from the pylorus. In the lumen of the small intestine, at the site of origin of these tumours, Dodd reported cauliflower-like growths. Webster found such outgrowths only occasionally, while McDonald and Leaver recorded thickening of the mucosa and submucosa at the site of the primary lesions but noted no polyps. The wall of the small intestine at the site of primary tumours was thickened, and the muscular layer was surrounded by an annular white fibrous lesion generally two to five centimetres in length, though in some cases encompassing a much greater length of the small intestine. Occlusion of the lumen of the small intestine at the primary site was accompanied by distension proximal to that point. Firm, white, scirrhus lesions, either continuous with the primary, or as isolated spots or plaques, were present on the mesentery encompassing the mesenteric lymph nodes in some cases, and along the serosa of the

intestinal tract. Secondary lesions also occurred on the abomasum, fore-stomachs, omentum, caecum, coiled colon and diaphragm. Metastatic nodules have been seen in the liver of some animals, and Webster reported two cases involving the splenic pulp. Pleural lesions were recorded by Webster, and metastatic lung lesions by McDonald and Leaver.

Dodd and McDonald and Leaver described the histopathology of these lesions. The appearance of the primary lesions was that of scirrhous adenocarcinomas involving mucus-secreting cells. The luminal surface of the lesions showed necrosis and ulceration, with the presence of a considerable number of neutrophils especially within acinar spaces. McDonald and Leaver considered mitotic figures were numerous. They also noted occasional infiltration of the internal muscle layer of the small intestine, and distension of lymphatic spaces between the internal and external muscle layers, by neoplastic tissue. Serosal lesions consisted of dense fibrous tissue containing relatively few carcinomatous cells.

In the liver, secondary lesions showed acinus formation with relatively little fibrous stroma. Numerous epithelial cells were present in arterial emboli near the fibrous pulmonary lesions described by McDonald and Leaver. The presence of numerous malignant cells in subcapsular and intermediate sinuses of lymph nodes draining affected areas was noted by Dodd.

The Pathology of Small Intestinal Carcinomas in Other Domestic Species

The pathology of small intestinal carcinomas in the domestic species other than sheep is described only on the basis of relatively few lesions.

In dogs, carcinomas of the small intestine have been diagnosed more commonly in the jejunum than in other sites. Of eleven small intestinal carcinomas listed by Krook (1956), nine originated in the jejunum, one in the duodenum and one in the ileum. Case et al. (1968) reported two cases arising in the duodenum. Eight of the cases discussed by Krook were annular, stenosing lesions. Histologically, six were classified as adenocarcinomas, three as carcinomas and two as "carcinoma gelatinosum" or mucoid carcinomas. Four of the lesions were scirrhus in nature; the others had a relatively scanty stroma. Metastases have been recorded in mesenteric, bronchial and hepatic lymph nodes, lung, myocardium and liver and on the peritoneum (Krook, 1956; Case et al., 1968).

From the limited number of cases described, carcinomas of the small intestine of cats appear to arise more commonly in the distal half of the small intestine, to be scirrhus in nature and to result in constriction of the intestinal lumen (Taylor and Kater, 1954; Theran and Thornton, 1968).

All twelve carcinomas of the small intestine of cattle reported by Misdorp (1967) had arisen in the jejunum. Metastatic lesions in cattle have been noted on the mesentery, in the liver, lungs (Schlegel, 1925) and mesenteric and mediastinal lymph nodes (Misdorp, 1967).

The Histogenesis of Experimentally Induced Carcinomas
of the Small Intestine

The histogenesis of adenocarcinomas of the small intestine produced experimentally in mice by oral dosing with 1, 2, 5, 6-dibenzanthracene or 20 methylcholanthrene was described by Stewart and Lorenz (1941a). The earliest lesions were "hyperplasia" and down growth of a number of the basal glands of the small intestinal mucosa into the lamina propria and the submucosa. Cells lining the infiltrating glands became atypical and sometimes several layers thick. Common cytological characteristics were intracytoplasmic mucus droplets and loss of cellular polarity. There were atypical hyperplastic changes in the superficial mucosa, with small areas developing irregular acini of small hyperchromatic cells. Increased fibrous tissue in the submucosa constricted, and ultimately obliterated, submucosal lymph vessels. As the stroma became more compact, the luminal surface was depressed below the level of the surrounding mucosa and the internal muscle layer became thickened centripetally. With the replacement of the mucosa and submucosa in the area of involvement, and the infiltration of muscle layers and the serosa by epithelial cells, the picture of an easily recognised carcinoma was complete.

These carcinomas spread to involve the mesentery, mesenteric lymph nodes and pancreas. Polypoid carcinomas were rare, as also were lesions commencing at the site of Peyer's patches. Most tumours originated 15 - 20 cm from the pylorus, which is approximately mid-length in the mouse's small intestine.

The earlier reports of ovine small intestinal carcinomas differed in some respects in their descriptions of lesions, and left unanswered a number of questions, such as the method and direction of spread of these tumours. In this section, the pathology and secondary spread of carcinomas of the small intestine of sheep are described, and these features are compared with those of man and other animals. Results of ultrastructural studies which clarify to some extent the histogenesis of these lesions are presented.

MATERIALS AND METHODS

With the exception of five sheep submitted for routine necropsy and found to have carcinomas of the small intestine, all lesions examined in this study were collected between 1966 and 1969 from ewes killed at ten Meat Export Works in New Zealand. The pathological description of lesions is based on gross examination of 450 of these neoplasms, 200 of which were examined histologically. Information on the number of primary lesions in each case, and the site of origin of tumours within the intestine, was obtained from a series of fifty consecutive cases examined during 1969.

For histopathology, tissues were fixed in 10% formol saline or Zenker's formol solution. Paraffin sections were stained with haematoxylin and eosin (H & E) and some were stained with periodic acid-Schiff (P.A.S.), van Gieson, alcian blue, Gomori's elastin, Weigert's elastin, Perls' iron and Mallory's trichrome stains. Serial sections were examined from selected blocks. Tissue from seven intestinal carcinomas of varying sizes was collected for electron microscopy following evisceration of ewes at a Meat Export

Works. The time from slaughter by exsanguination to collection of material was approximately 10 minutes. For comparative purposes, mucosa was similarly obtained from four normal sheep. Following primary fixation for 3 - 4 hours in a solution of 2% glutaraldehyde and 3% formaldehyde in 0.1 Molar phosphate buffer, pH 7.2, at 4°C, tissues were washed twice in phosphate buffer, pH 7.2, and placed in a secondary fixative of 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, at 4°C for 3 - 4 hours. Blocks of tissue were then washed again in buffer and dehydrated in a graded series of ethanol, being left overnight in 75% ethanol containing 1% uranyl acetate. Specimens were next infiltrated by a propylene oxide-epoxy resin series (Luft, 1961) with epoxy resin (Durcupan - ACM*) and embedded in pure resin in number 4 gelatin capsules and polymerised for 48 hours at 60°C. "Pale gold" sections cut on an LKB "Ultratome II" and stained with aqueous uranyl nitrate followed by lead citrate (Reynolds, 1963), were examined with a Philips EM200 electron microscope.

Sections of epoxy resin embedded tissues were also cut at 2 μ and stained with haematoxylin and phloxine (Shires, Johnson and Richter, 1969) for examination by light microscopy.

RESULTS

Gross Pathology of Small Intestinal Carcinomas

In this series, primary lesions of intestinal carcinomas were not noted in the duodenum but occurred at all levels of the jejunum and ileum with the exception of the most proximal portion of the jejunum.

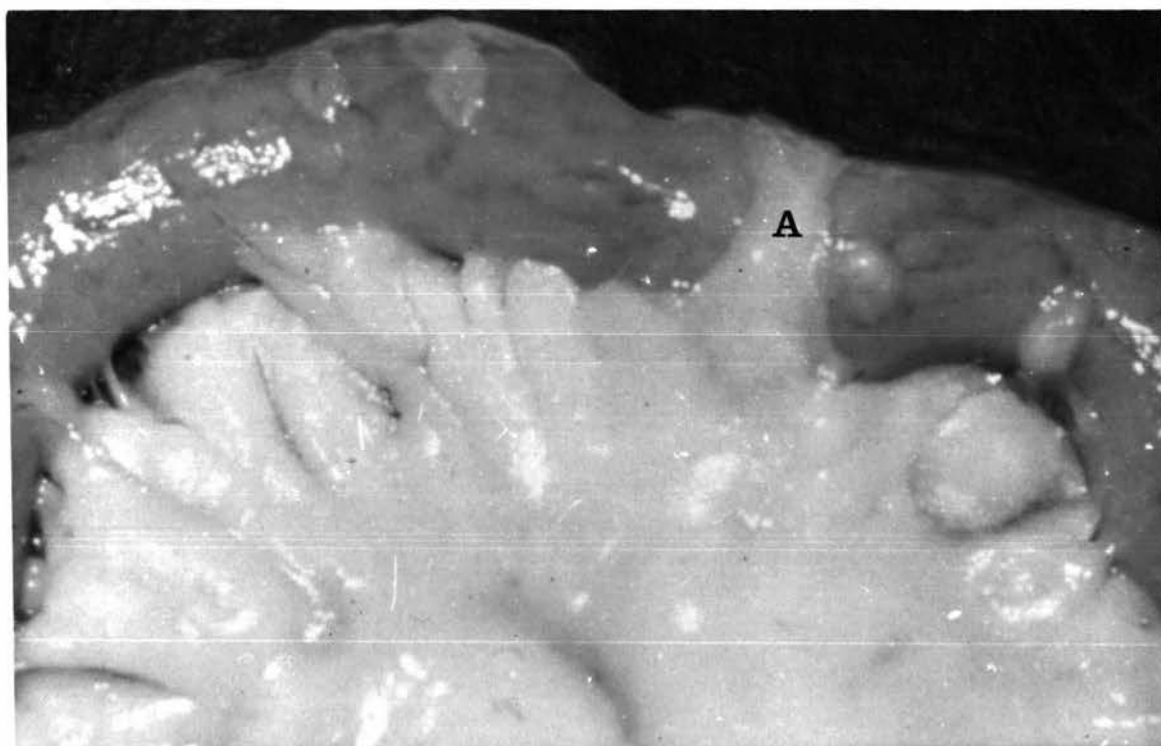
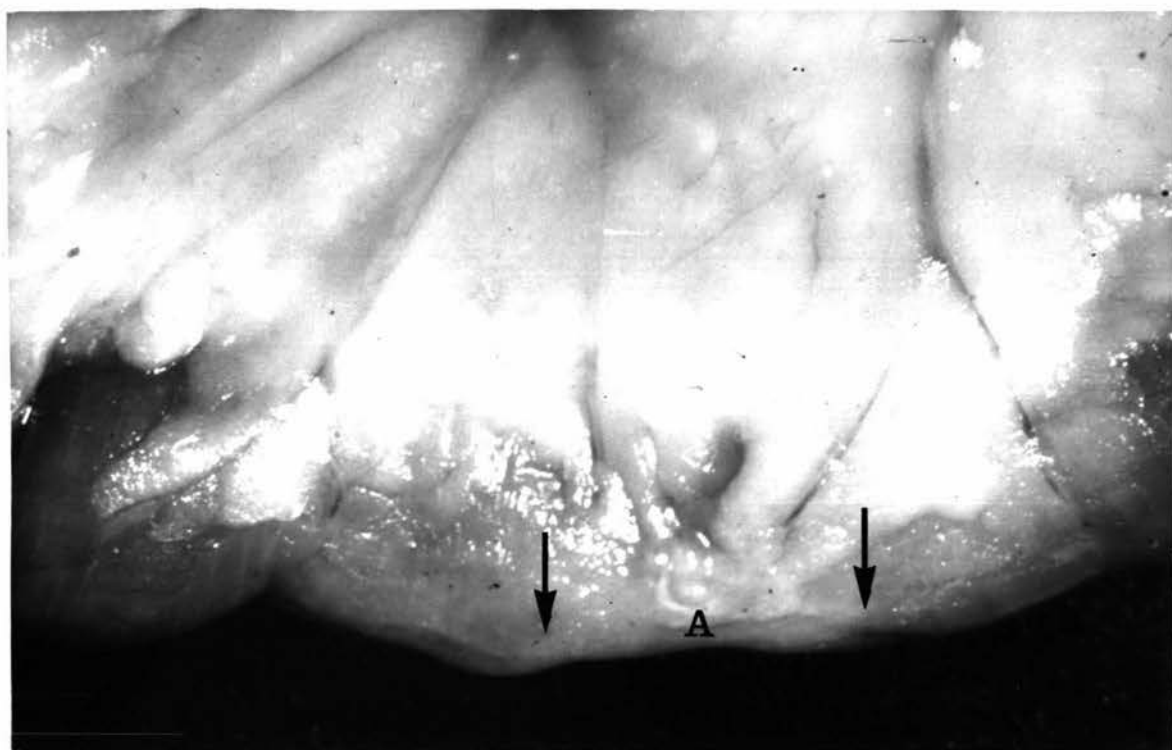
* Fluka A.G., Buchs, Switzerland

Figure 2

A carcinoma of the small intestine appearing as a small white area on the intestinal serosa (A) but visible through the serosa over a larger area (limits indicated by large arrows).

Figure 3

A carcinoma of the small intestine (A) extending as an annular band around the intestine.



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Figure 4

The luminal surface of a small intestinal carcinoma (C) raised above the surrounding mucosa. (scale is in cm).

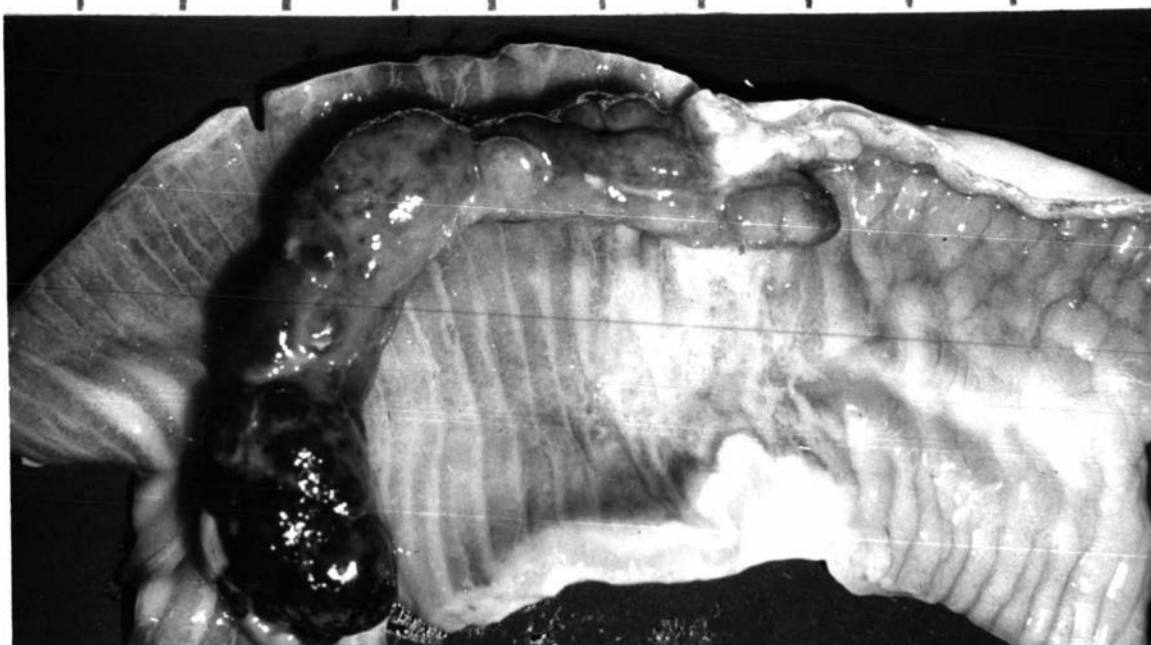
Figure 5

A carcinoma of the small intestine with a single large polyp attached to the mucosal portion of the primary lesion (scale is in cm).



1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10

1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10



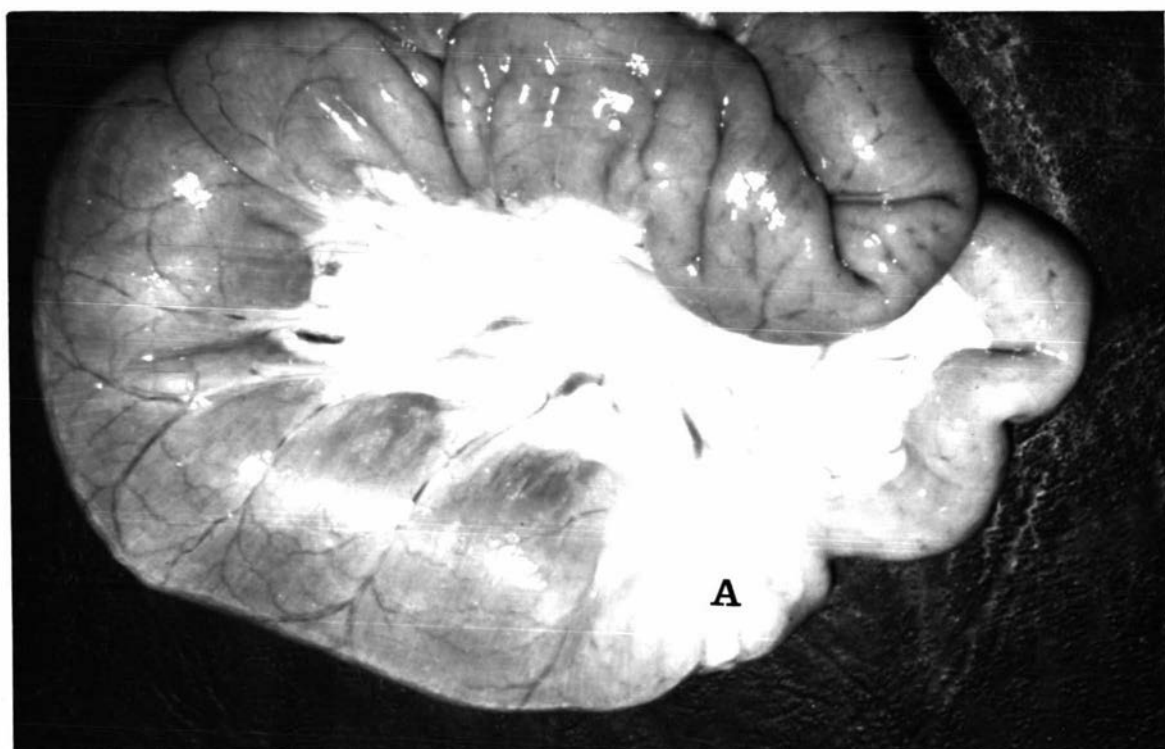
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Figure 6

Multiple polyps (P) developing from the luminal surface of a primary carcinoma of the small intestine (scale is in cm).

Figure 7

Dilatation of the small intestine proximal to the primary lesion of a small intestinal carcinoma (A).



T A B L E X I

The Number of Primary Lesions in Each of Fifty Consecutive
Sheep with Carcinomas of the Small Intestine

<u>Number of primary lesions</u>	<u>Number of sheep</u>
1	44
2	3
3	Nil
4	3

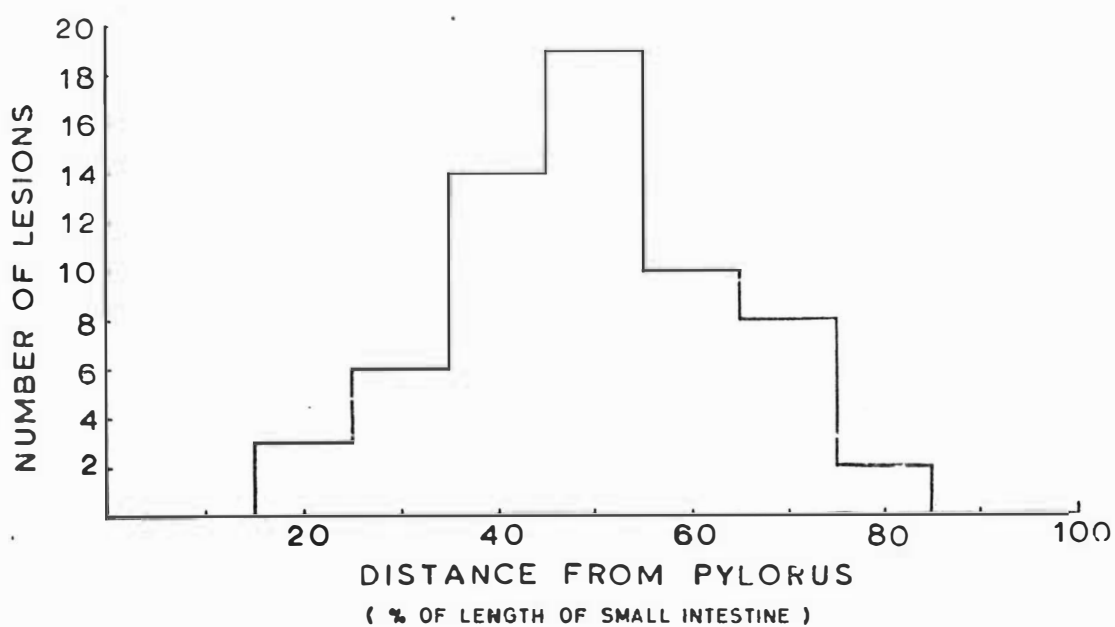


Figure 1

The anatomical distribution of primary lesions of small intestinal carcinoma in fifty consecutive affected sheep.

Figure I shows the distribution of primary lesions in fifty consecutive cases. Apparent multiple primary tumours of the small intestine were not uncommon, being found in six of these cases (Table XI). Though not encountered in this series of fifty cases, carcinomas have also been noted in the more distal portions of the small intestine, and five carcinomas of the coiled portion of the large intestine were found during these investigations.

The smallest primary lesions found presented as light pink areas 0.3 - 0.5 cm in diameter, visible through the intestinal serosa. These lesions which, histologically, involved the mucosa, submucosa and muscularis of the intestinal wall were firmer than normal intestine, and their luminal surface was smooth and slightly raised above the surrounding mucosa.

Other small primary lesions of intestinal carcinomas presented as firm, white lesions, 0.1 cm or more in diameter, on the serosal surface of the small intestine (Figure 2). Larger lesions extended as incomplete or complete annular bands of tissue (Figure 3), partially constricting the wall of the small intestine. The mucosal surface of some primary lesions was ulcerated and depressed beneath the level of the surrounding tissue. More frequently, the mucosa, submucosa and internal muscular layers were centripetally thickened beneath the annular serosal lesion, with the affected luminal surface appearing as a well defined annular band, firmer than normal and raised above the surrounding mucosa (Figure 4). In approximately 20% of lesions, one to seven polyps protruded from the affected mucosa into the intestinal lumen (Figures 5 and 6). Partial occlusion of the lumen occurred in many cases, resulting in proximal dilatation

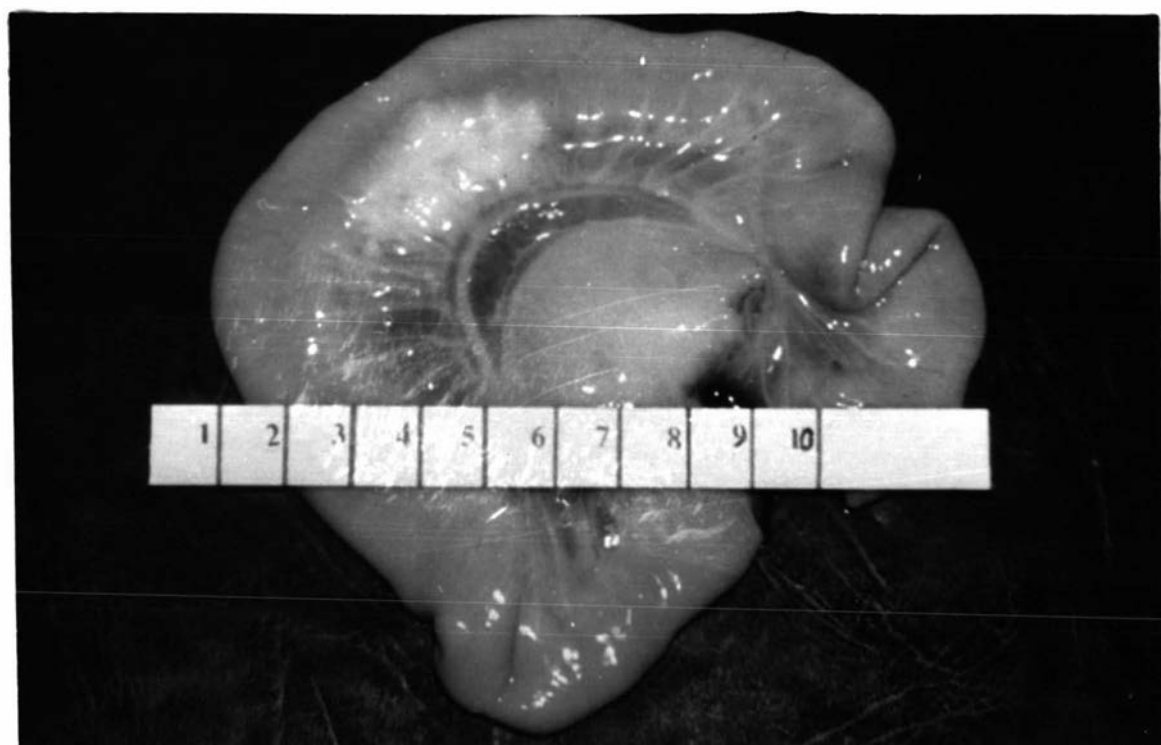
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Figure 8

White threads of lymphatic permeation (LP) extending from a carcinomatous lesion on the intestinal wall (I) to an affected mesenteric lymph node (LN).

Figure 9

Secondary lesion of a small intestinal carcinoma at the mesenteric attachment of the small intestine (scale is in cm).



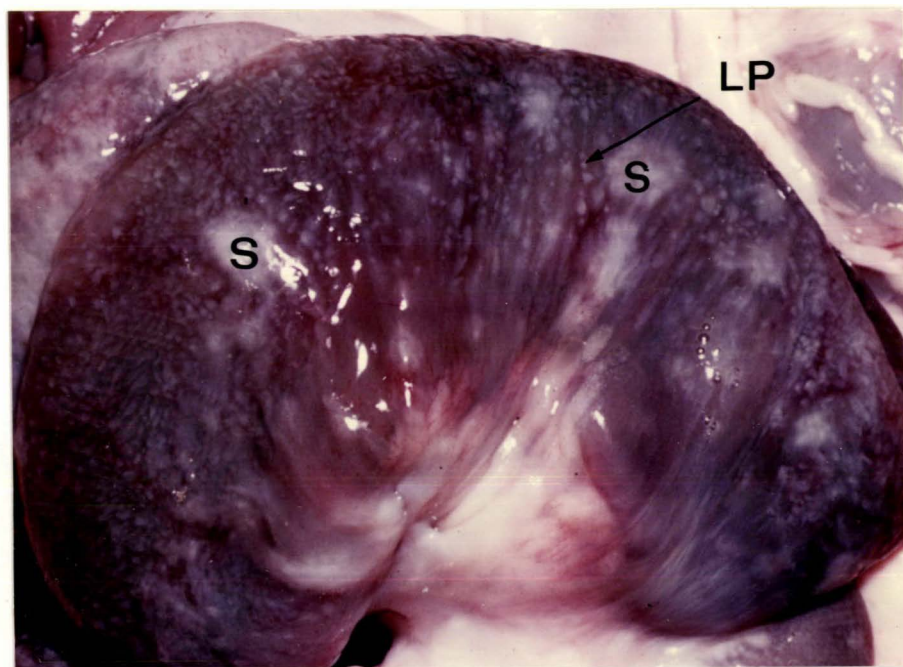
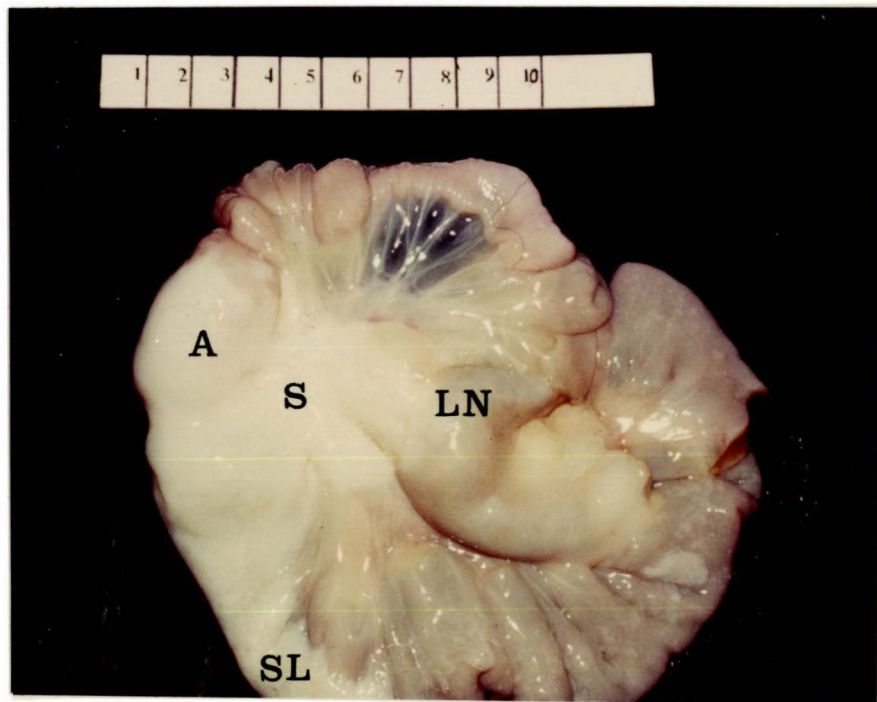
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Figure 10

Primary lesion (A) of a small intestinal carcinoma with spread proximally along the intestinal serosa (SL) and with a band of scirrhous tissue (S) extending across the mesentery to a mesenteric lymph node (LN). (scale is in cm)

Figure 11

Part of small intestine proximal to primary carcinomatous lesion with small nodules (S) on serosal surface with threads of lymphatic permeation (LP) between the nodules and the mesenteric attachment of the small intestine.



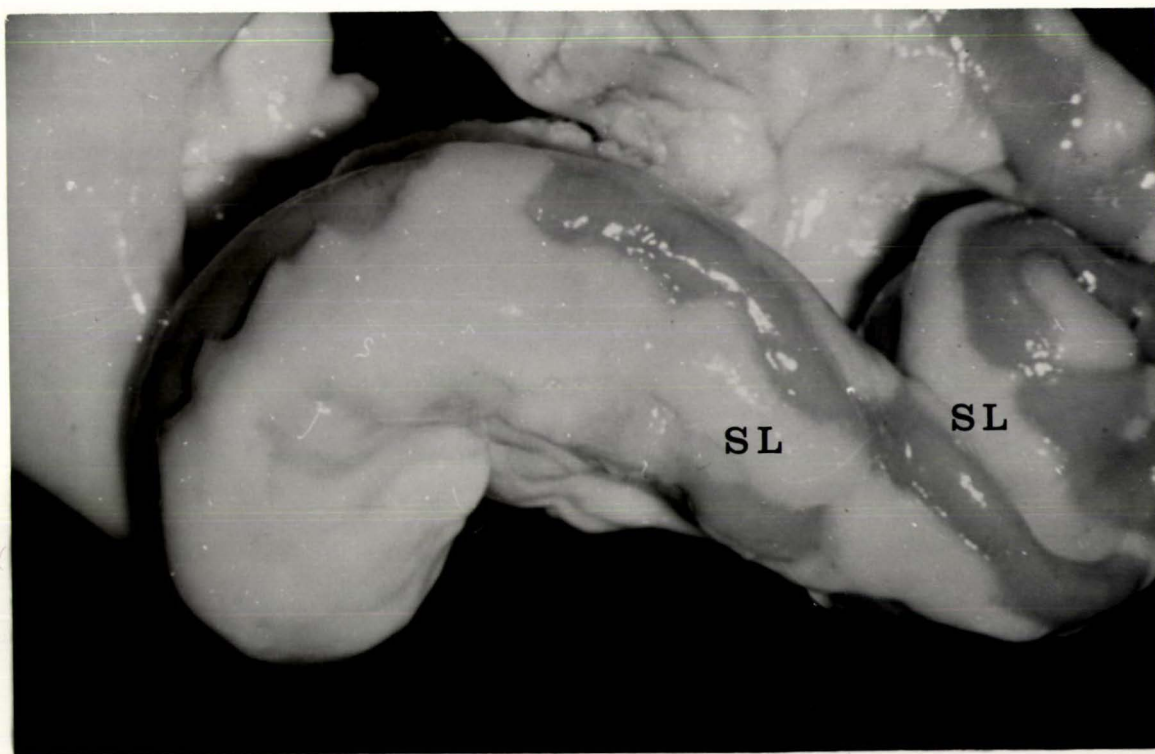
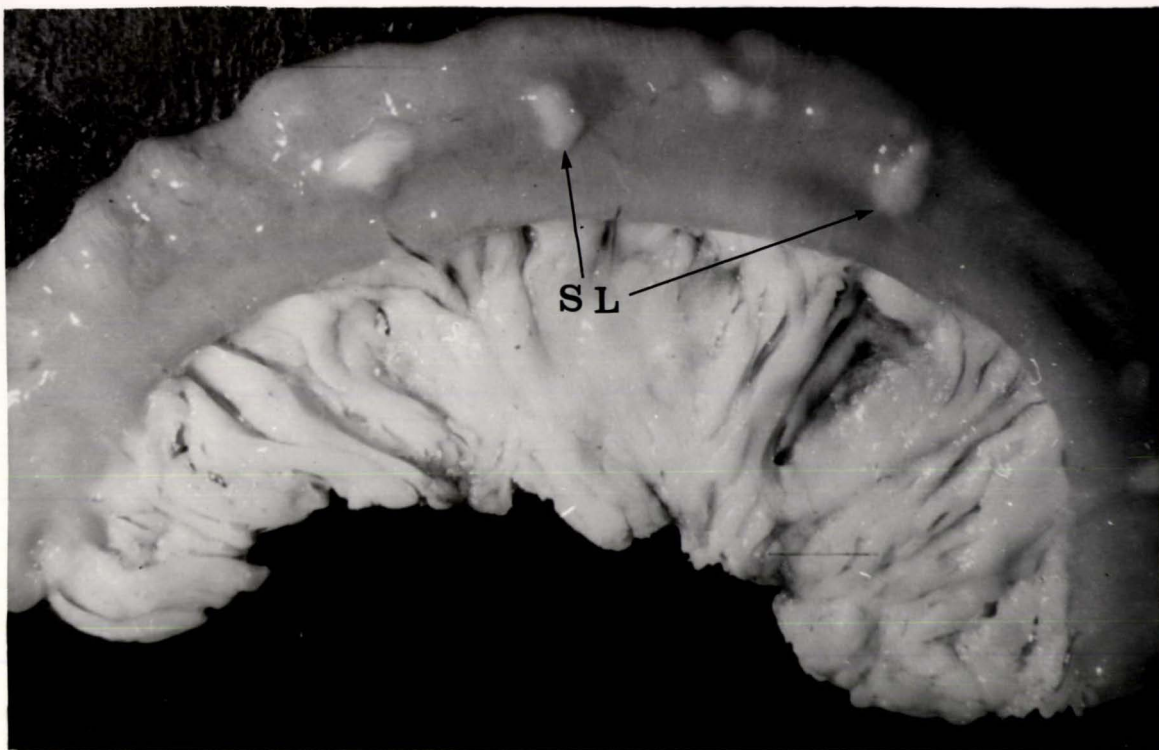
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Figure 12

Small secondary lesions (SL) of an intestinal carcinoma,
located on the wall of the small intestine.

Figure 13

Bands of scirrhous neoplastic lesions (SL) extending
longitudinally along the wall of the small intestine.



of the intestine (Figure 7).

Examination of more widely disseminated tumours indicated that they spread from the primary lesion, described above, in a relatively constant and predictable manner. In the mesentery of some sheep, there was growth toward the related mesenteric lymph node in the form of fine white threads, that were interpreted as representing permeation of neoplastic tissue within lymphatics (Figure 8). In some cases, discrete white nodules were found along the line of lymphatics, while in others a broad band of white scirrhous tumour tissue extended in the mesentery toward the lymph node (Figure 10).

Lesions also occurred along the outside of the intestinal wall at its mesenteric attachment, predominantly in a proximal direction from the primary. Although often appearing as a continuous strip of tumour tissue, they, at other times, occurred as focal lesions in this location (Figure 9). Numerous small nodules of tumour were frequently noted along the serosa on the sides of the intestinal wall. Some of these could be seen to be expansions from thread-like growths of tumour running beneath the serosa across the intestine from its mesenteric attachment (Figure 11). Most lesions along the proximal portion of the small intestinal wall, however, were isolated spots or small plaques (Figure 12), and histological sections around these lesions failed to reveal evidence of contiguous spread. From the primary tumour these focal lesions extended proximally, sometimes as far as the pylorus. Spread in the distal direction was usually very limited. Secondary lesions were more numerous and larger near the primary lesions, and in some cases had coalesced to form two bands extending longitudinally on the lateral portions of the intestinal

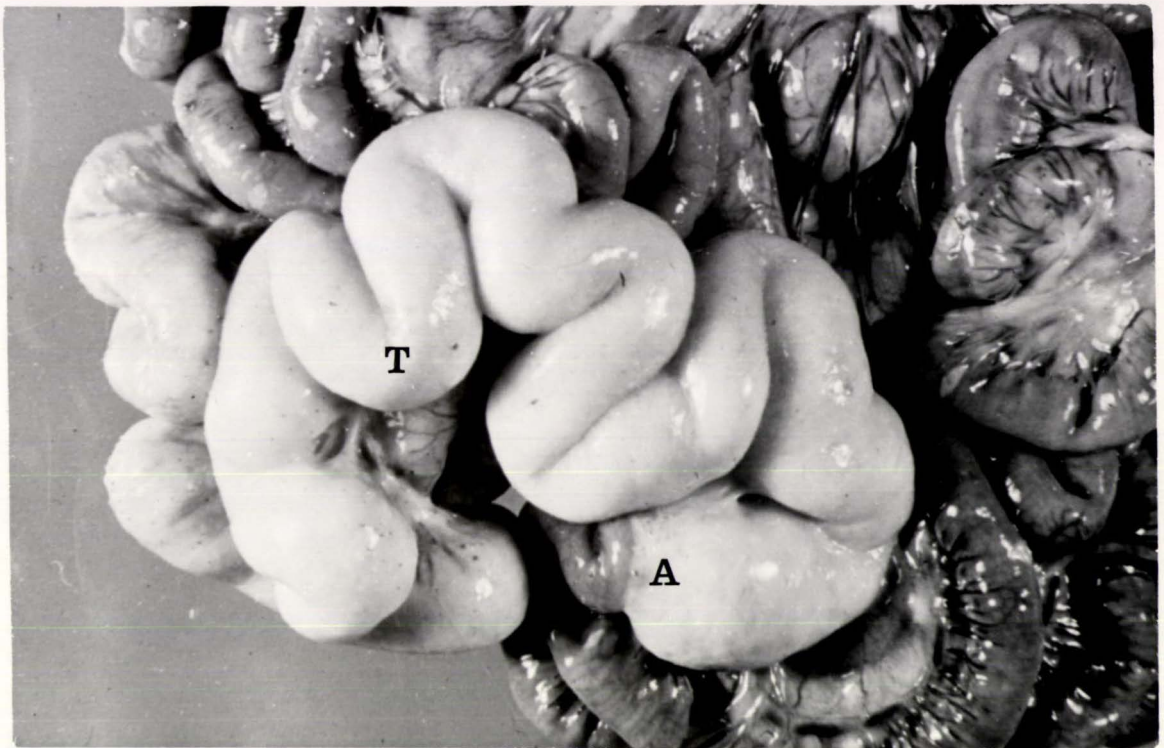
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Figure 14

Scirrhus tumour tissue (T) ensheathing a length of small intestine proximal to the primary lesion of an intestinal carcinoma (A).

Figure 15

Translucent lesion (Tr) on the serosal surface of the intestine proximal to the primary lesion of a carcinoma of the small intestine (scale is in cm).



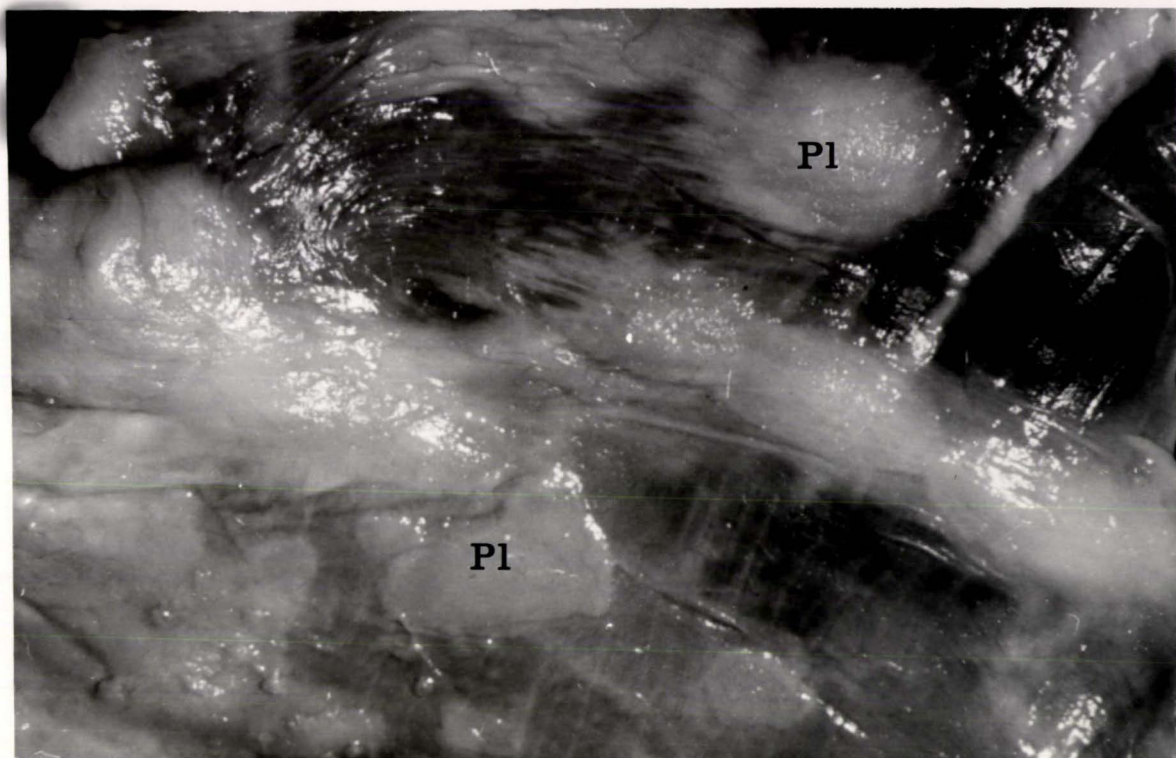
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Figure 16

Secondary lesions of a carcinoma of the small intestine forming white plaques (Pl) on the parietal peritoneum.

Figure 17

Secondary lesions from a small intestinal carcinoma involving the diaphragm.



following page 32

Figure 41

Neoplastic cells (NC), some of which contain mucus (Mu), in the subcapsular sinus of a mesenteric lymph node. P.A.S. x 400.

Figure 42

Neoplastic cells (NC) in the intermediate sinuses of a mesenteric lymph node. H & E. x 400.

wall (Figure 13). Considerable lengths of the small intestine were totally ensheathed in scirrhus tumour tissue in some specimens (Figure 14). In very advanced cases, lengths of small intestine, mesentery and mesenteric lymph nodes and portions of the large intestine were sometimes included in a considerable mass of dense, white, scirrhus tumour tissue, arising, presumably, by extension and coalescence of smaller lesions.

Frequently, cases with marked distension of the intestine proximal to the primary lesion showed a soft, pink, translucent layer up to 1.0 cm thick on the serosa of the dilated intestine (Figure 15).

Secondary lesions were also found on the serosal surfaces of the colon, caecum, abomasum, forestomachs, mesentery, omentum, spleen, liver, diaphragm, parietal peritoneum (Figure 16) and parietal pleura. Lesions on the parietal peritoneum were irregular, dense, white, scirrhus plaques of various diameters and up to 0.5 cm thick. They were predominantly on the anterior and ventral portions and were often continuous with similar lesions on the abdominal surface of the diaphragm. Diaphragmatic lesions (Figure 17) were usually most pronounced about the junction of the muscular and tendinous portions of the diaphragm. Similar, but smaller, lesions were also seen in some animals on the thoracic surface of the diaphragm and on the parietal pleura. These latter often appeared as irregular ribbons of firm, white, scirrhus tissue adjacent to the anterior and posterior costal borders.

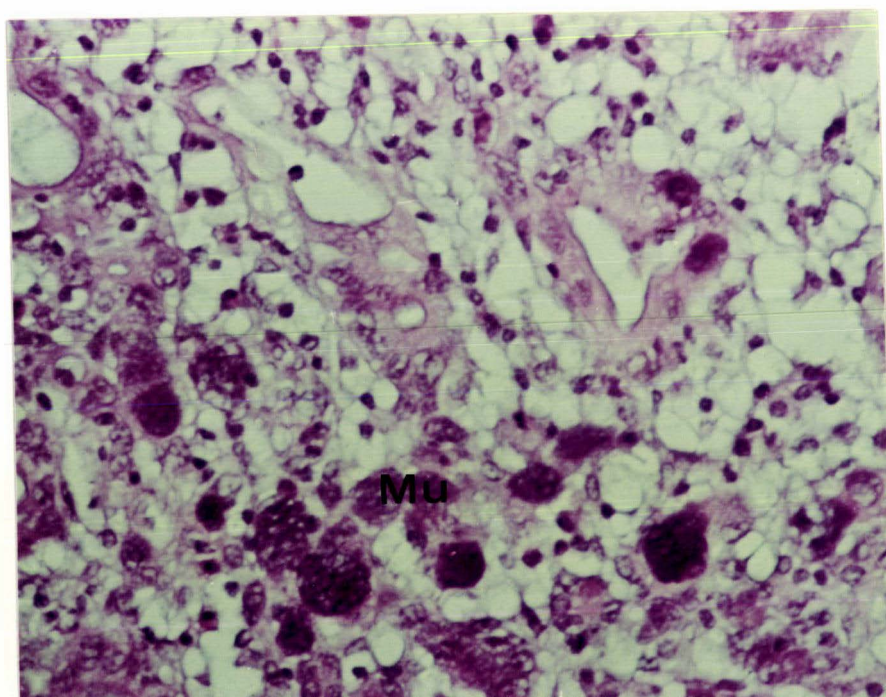
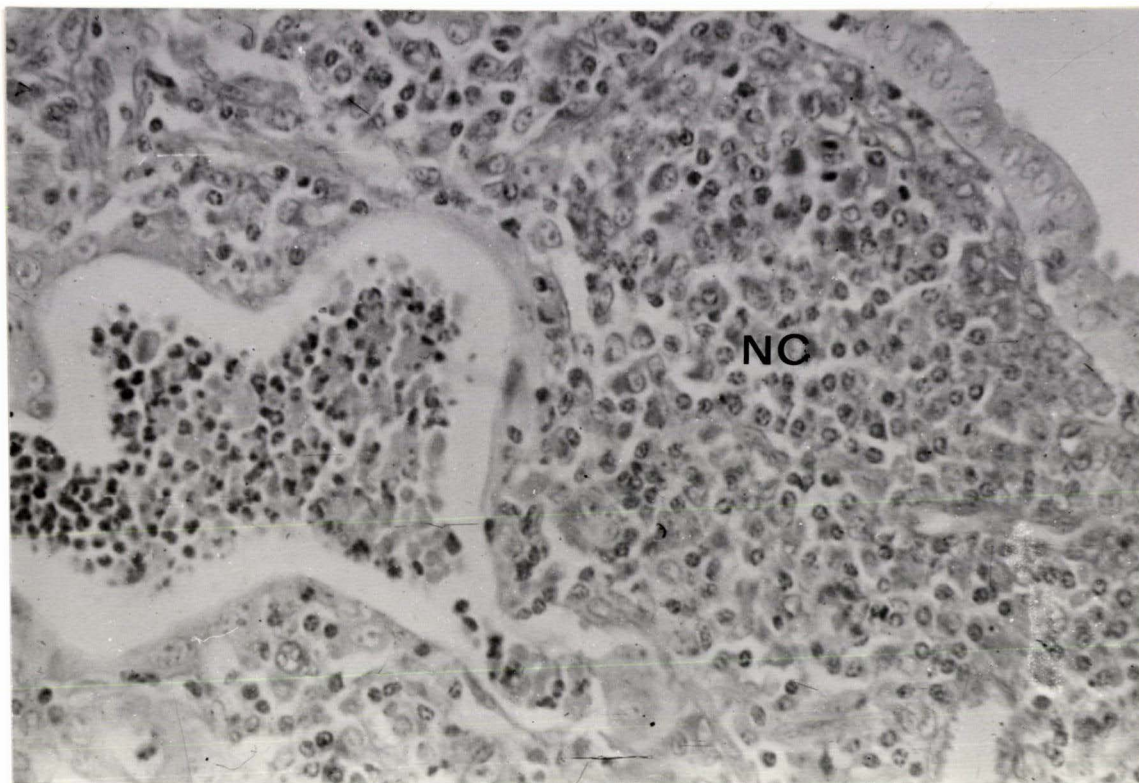
Only three sheep were noted as having secondary lesions in the hepatic parenchyma. These were multiple, firm, pink areas, varying in

Figure 18

Portion of carcinoma of the small intestine replacing the intestinal mucosa. The lesion consists, predominantly, of neoplastic cells (NC), and the intact luminal surface is lined by a single layer of cuboidal cells. P.A.S. x 150.

Figure 19

Portion of carcinoma of the small intestine replacing the intestinal mucosa. In one area none of the cells appear to be producing mucus, while in another many of the neoplastic cells contain mucus (Mu). H & E. x 500.



diameter from approximately 0.1 to 1.5 cm, scattered throughout the liver substance. In each case, there was widespread dissemination of lesions elsewhere, as described above.

The presence of fibrous tags and plaques on the liver capsule in some cases, and the involvement of hepatic lymph nodes in two of thirty sheep from which these lymph nodes were examined histologically, were the only other signs suggestive of liver involvement.

Mesenteric lymph node involvement was observed to accompany even the smallest of primary lesions, and was noted in some cases in which penetration of the neoplasm to the serosal surface of the intestine had not yet occurred. Affected nodes were white, enlarged and had a firm texture (Figure 10). Dorsal mediastinal lymph nodes were occasionally affected in animals that also had secondary lesions on the diaphragm and parietal pleura.

In four of the five sheep killed in the late stage of this disease, there were 10 to 20 litres of straw coloured ascitic fluid in the abdominal cavity.

Histopathology of Small Intestinal Carcinomas

Histological examinations of intestinal lesions showed them to be neoplasms of cells resembling either columnar epithelial cells or mucus-secreting goblet cells of the intestinal epithelium. These replaced the mucosa locally (Figure 18) and invaded the submucosal, muscular and serosal layers. In all lesions, some cells possessed the ability to produce mucus (Figure 19), though the proportion of neoplastic cells containing mucous vacuoles varied between tumours and

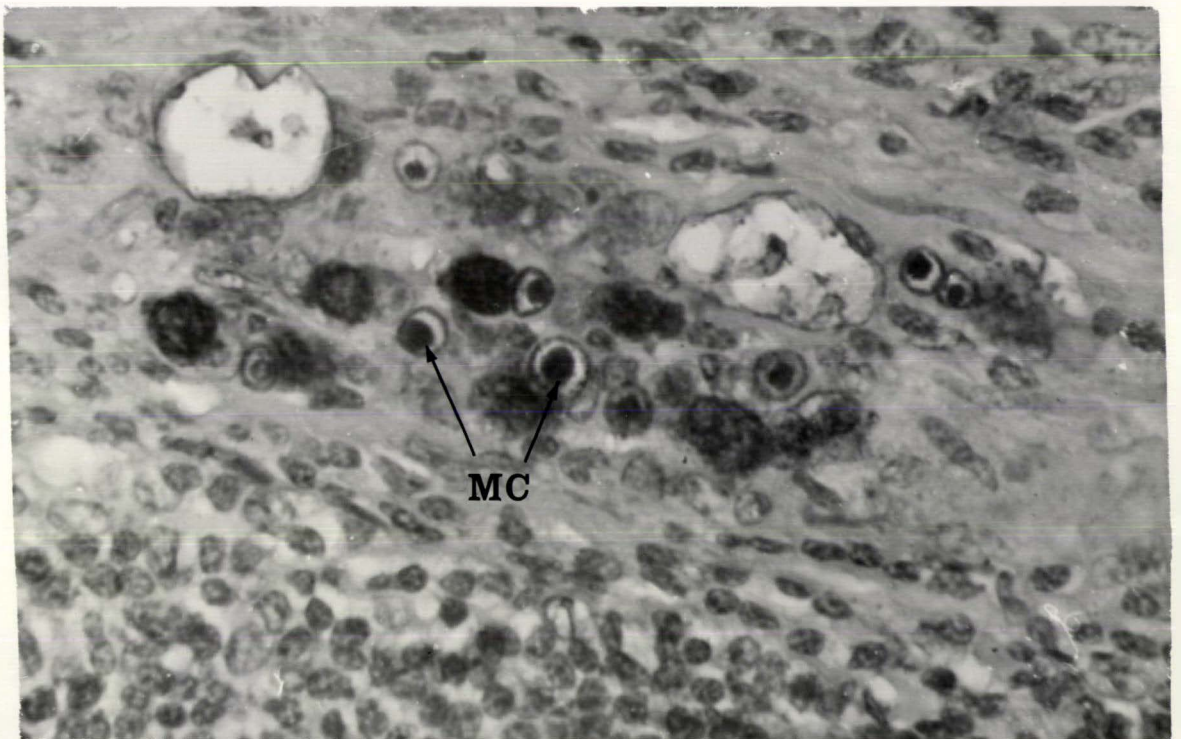
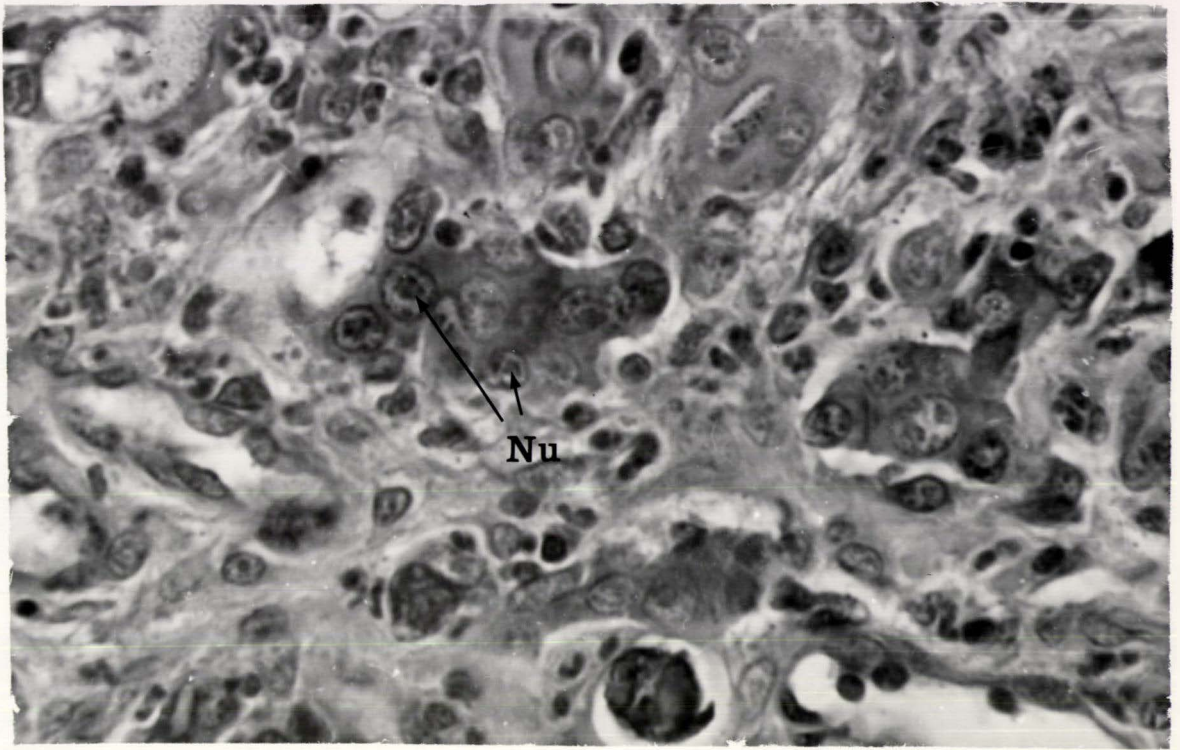
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Figure 20

Neoplastic cells in the mucosal region of small intestinal carcinoma. The nuclei contain either one or two large nucleoli (Nu).
H & E. x 900.

Figure 21

Structures which appeared as large basophilic intracytoplasmic inclusions (MC) in H & E stained paraffin sections. These proved to be intracytoplasmic microcysts (see figures 24, 54, 60 and 61) H & E.
x 900.



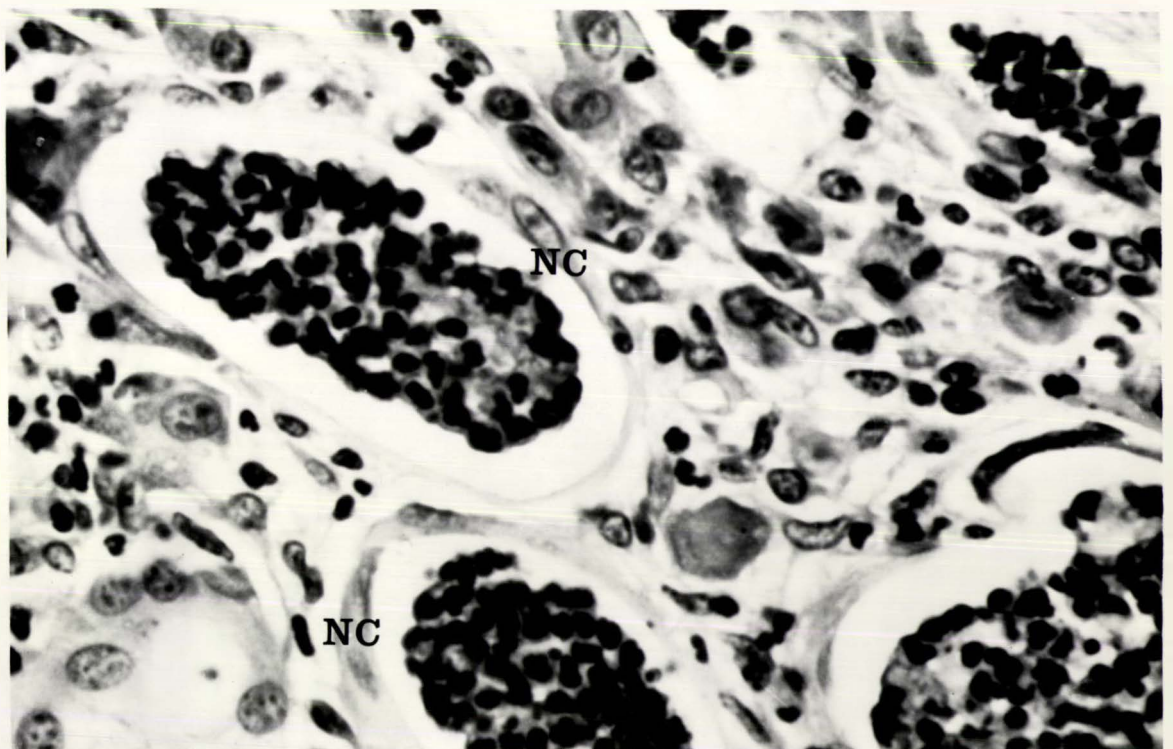
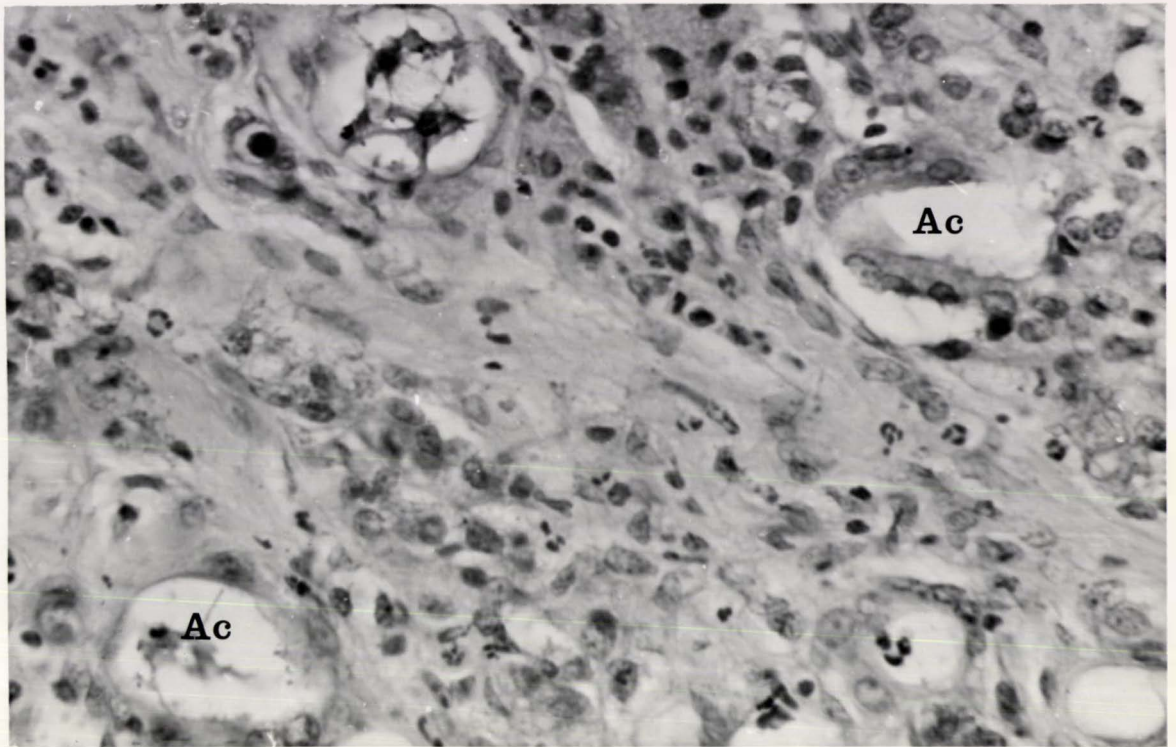
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Figure 22

Acinar arrangement (Ac) of neoplastic cells in that portion
of a small intestinal carcinoma replacing the mucosa. H & E. x 450

Figure 23

Acini, composed of flattened neoplastic cells (NC), and
containing numerous neutrophils. H & E. x 650



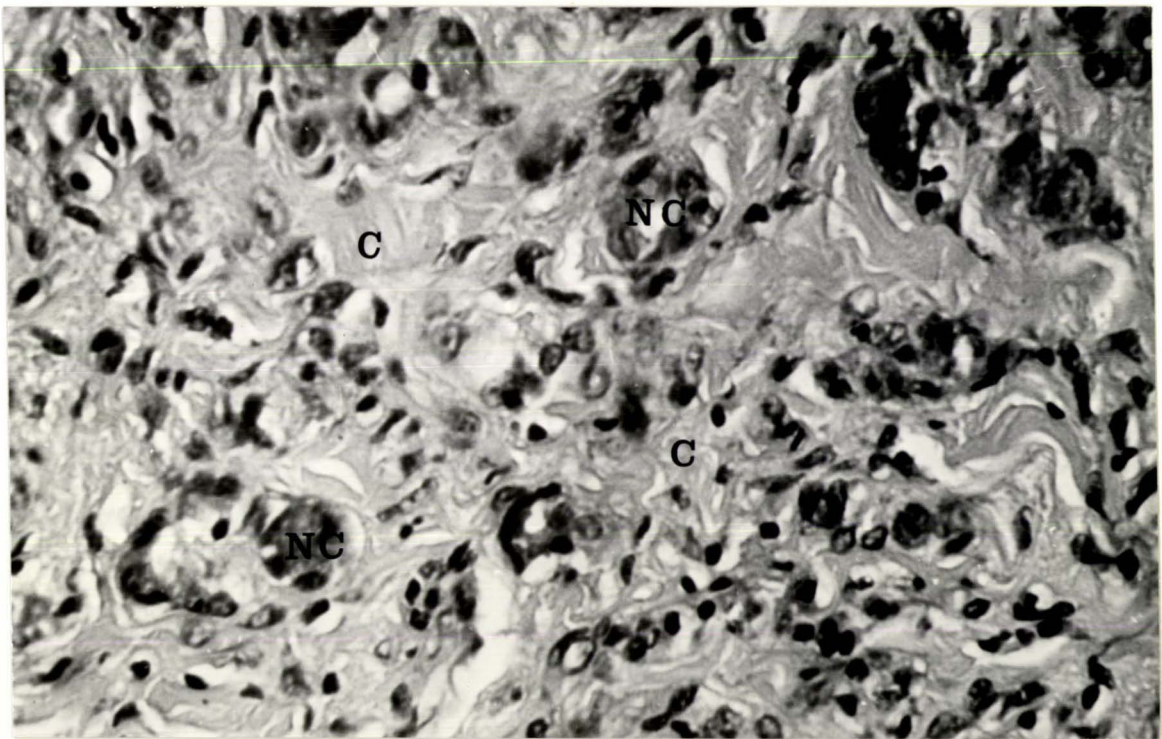
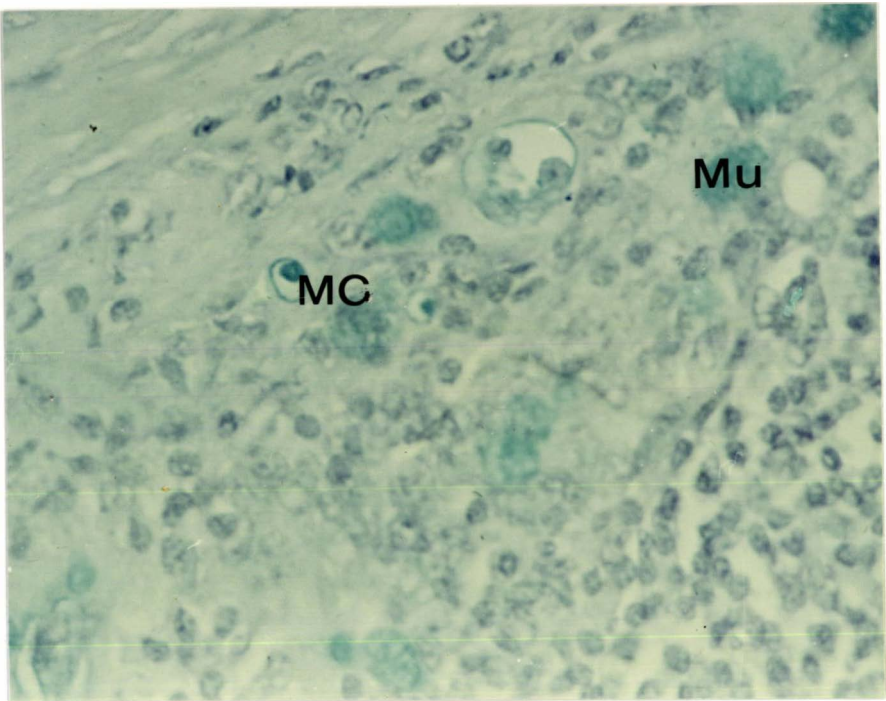
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Figure 24

Intracytoplasmic vacuoles (MC) with both contents and margins staining with alcian blue. (Mu = mucus in other neoplastic cells).
Alcian blue. x 550

Figure 25

Submucosal portion of a carcinoma of the small intestine with abundant collagenous stroma (C) between the neoplastic cells (NC).
H & E. x 400.



between parts of tumours. The nuclei of neoplastic cells contained one or two large nucleoli (Figure 20). Mitotic figures were infrequent. Acinar arrangement of cancerous cells was always present (Figure 22), but the proportion of cells forming acini varied between tumours and parts of tumours. The degree of nuclear polarity within the cells of acini also varied. In neoplastic tissue replacing the mucosa, there were often acini that were composed of slightly flattened tumour cells and which contained numerous neutrophils (Figure 23). Non-ulcerated portions of the luminal surface of neoplasms were covered with a single layer of cuboidal epithelium.

In the mucosal portion of lesions, and very occasionally elsewhere, large, round, basophilic (occasionally eosinophilic) bodies, approximately 5μ in diameter and surrounded by clear haloes were seen within the cytoplasm (Figure 21). With P.A.S. and alcian blue stains, both the central bodies and margins of these apparent vacuoles stained similarly to mucus (Figure 24). All lesions contained cells with these structures, though in varying numbers. Light microscopy of 2μ sections of epoxy resin-embedded material showed them to be intracytoplasmic vacuoles lined by narrow brush borders and containing amorphous material.

Within the mucosal portion of tumours, there was a moderate collagenous stroma. Considerable numbers of inflammatory cells, including eosinophils and plasma cells, were scattered diffusely throughout.

Commonly, the submucosa was thickened and largely replaced by tumour cells, in groups and acini, within a fibrous stroma, more plentiful than in the mucosal portion of lesions (Figure 25). Blood vessel walls and lymphatics showed invasion by neoplastic cells and

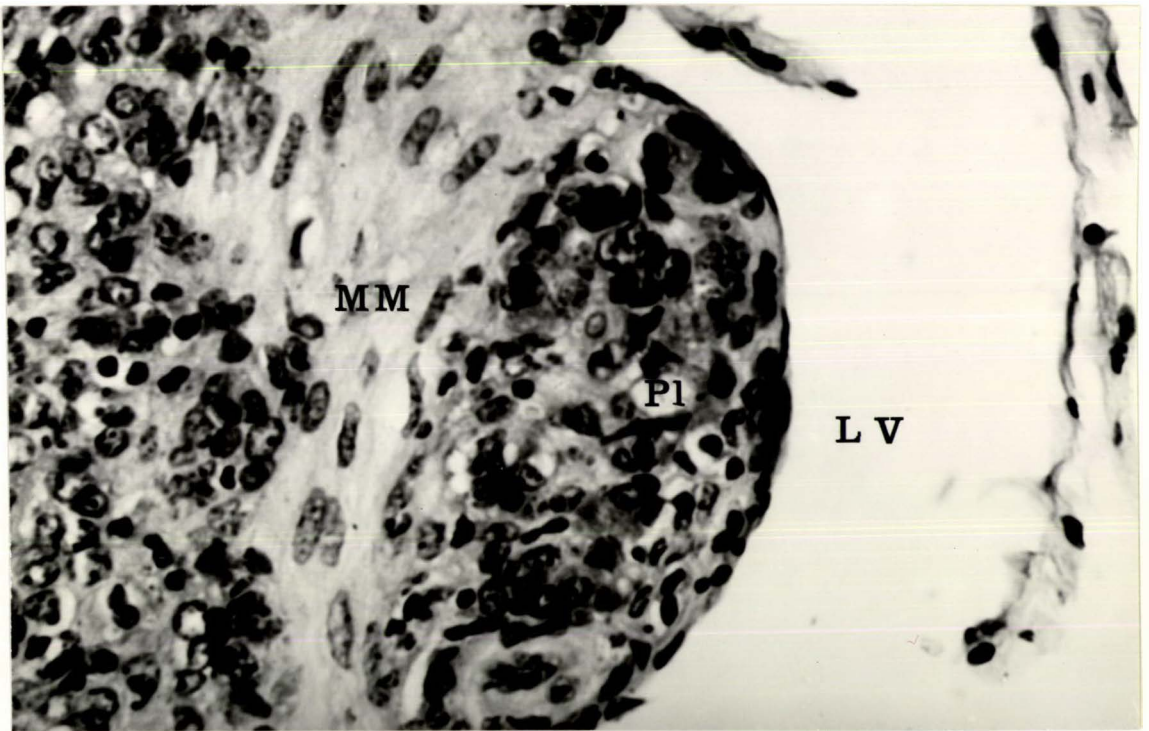
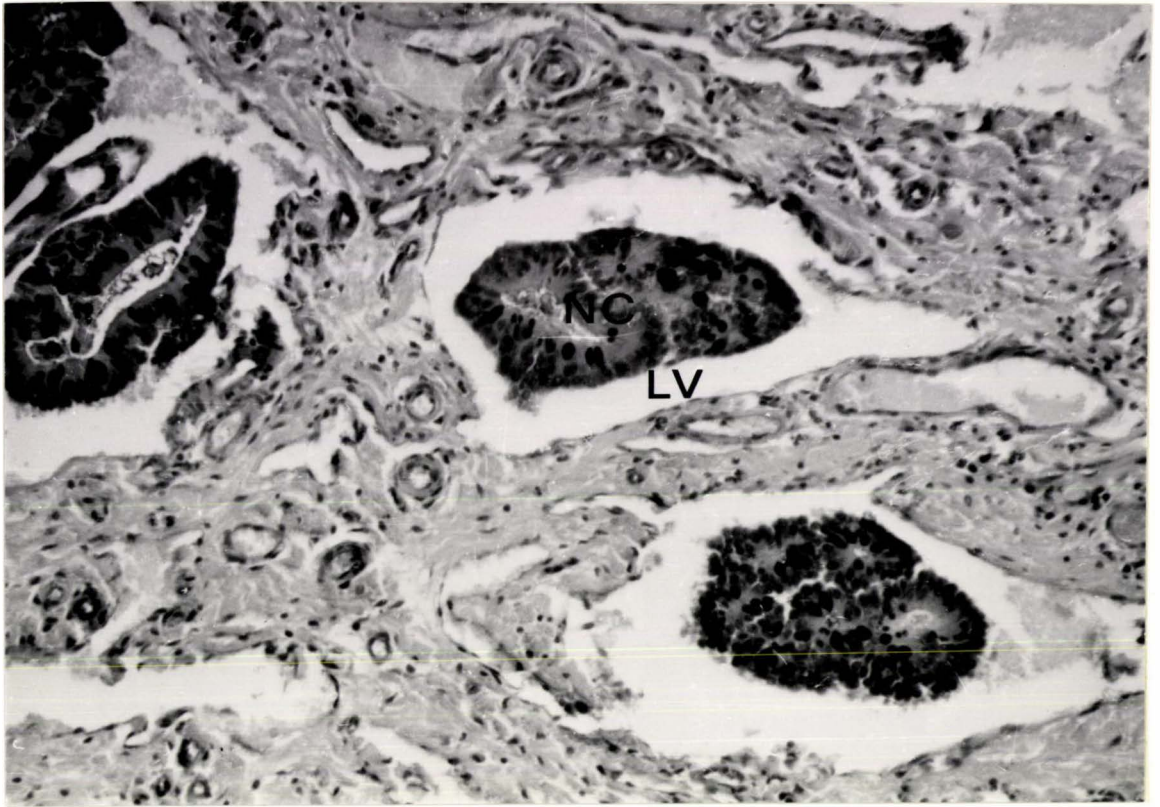
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Figure 26

Groups of neoplastic cells (NC) within lymph vessels (LV) of the lamina propria of the small intestine. H & E. x 150.

Figure 27

A plaque of neoplastic cells (Pl) protruding into the lumen of a lymph vessel (LV) immediately beneath the muscularis mucosa (MM) near the primary lesion of a small intestinal carcinoma. H & E. x 400.



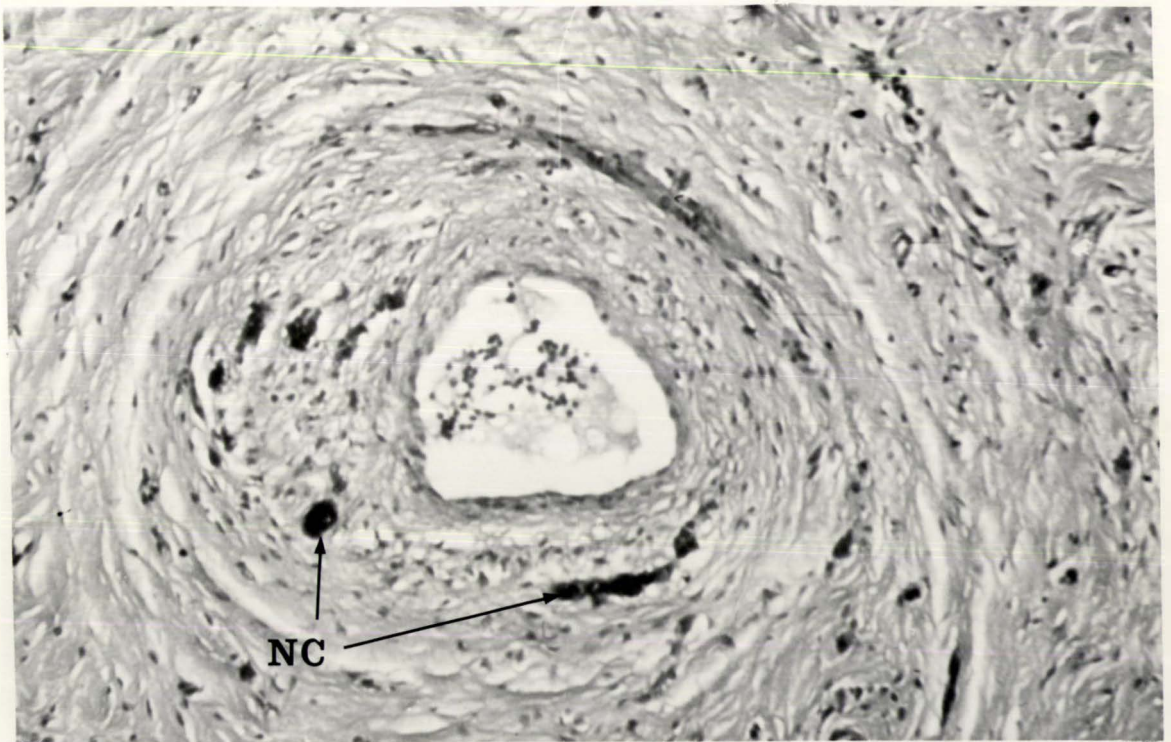
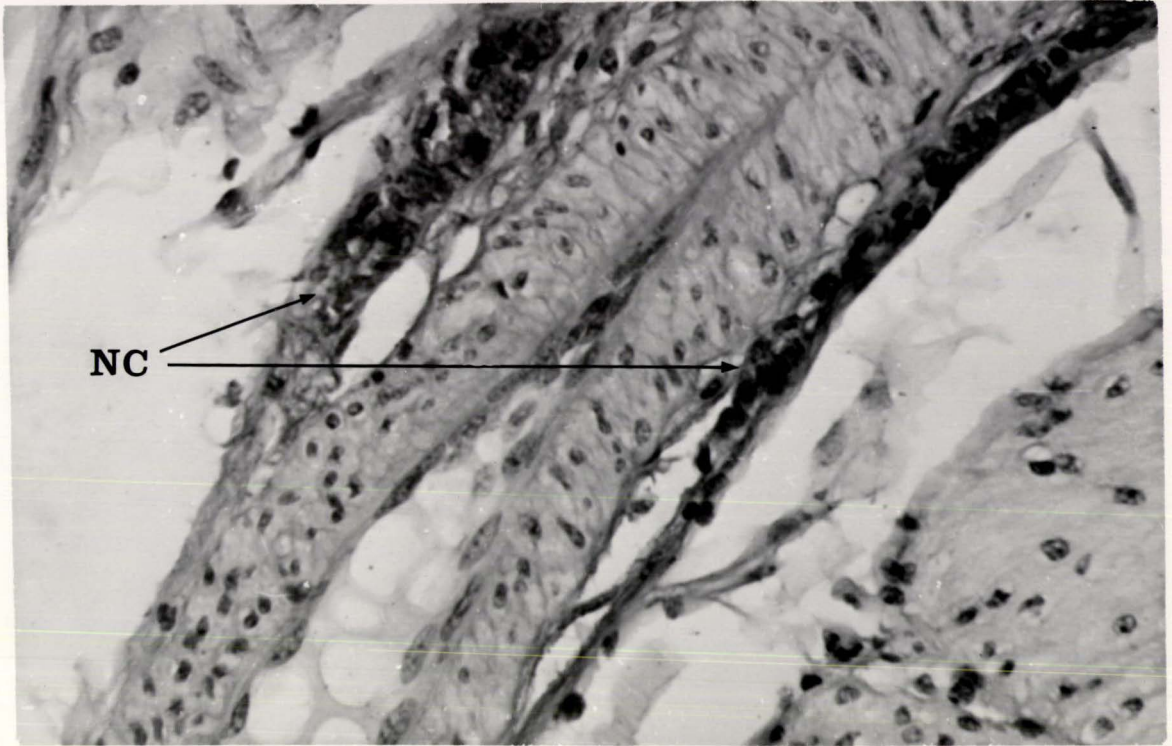
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Figure 28

Neoplastic cells (NC) in the the tunica adventitia of a
submucosal blood vessel. H & E. x 400

Figure 29

Neoplastic cells (NC) in the thickened wall of a blood
vessel. H & E. x 150.



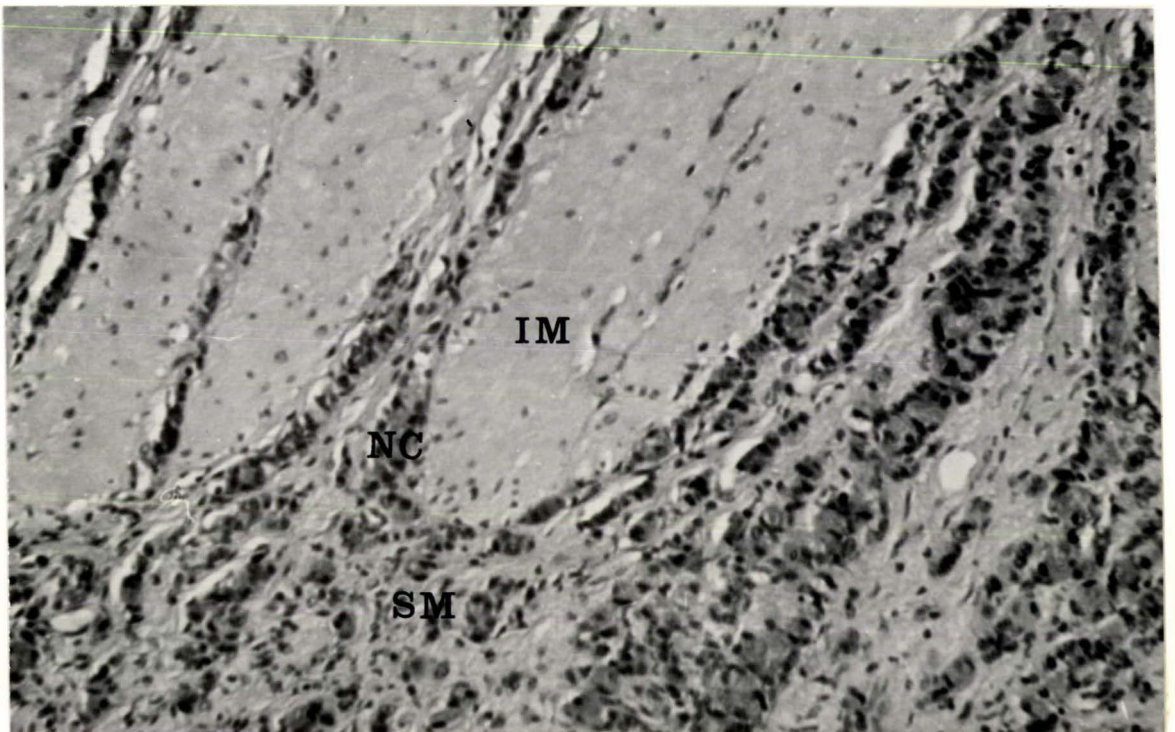
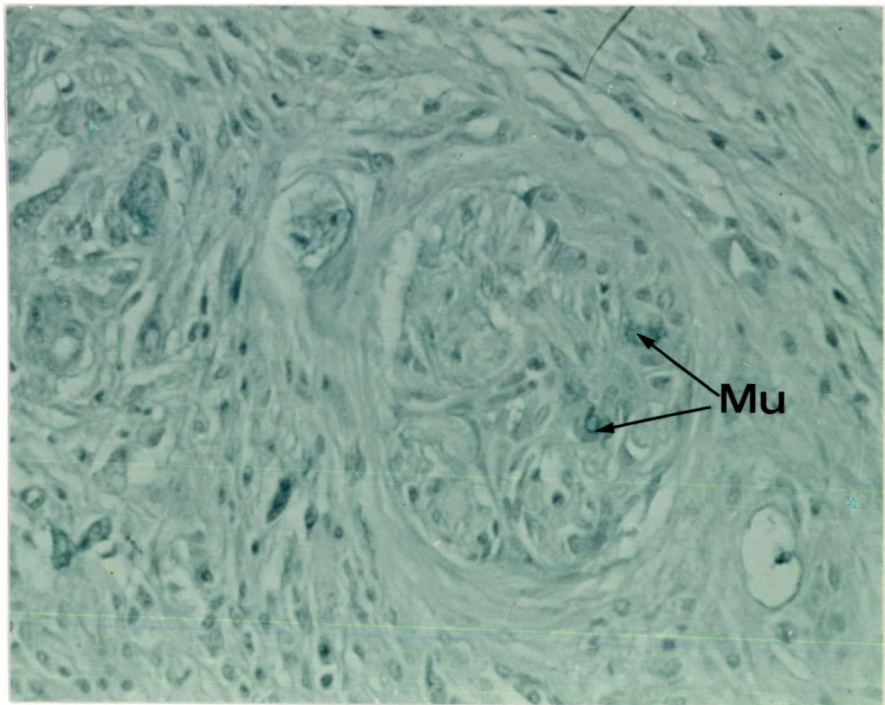
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Figure 30

Neoplastic cells containing mucus (Mu) within a nerve in the wall of the small intestine of sheep with a carcinoma of the small intestine. Alcian blue. x 350

Figure 31

Neoplastic cells (NC) penetrating from the submucosal portion of a carcinoma of the small intestine (SM) between muscle bundles of the internal muscular layer (IM). H & E. x 150



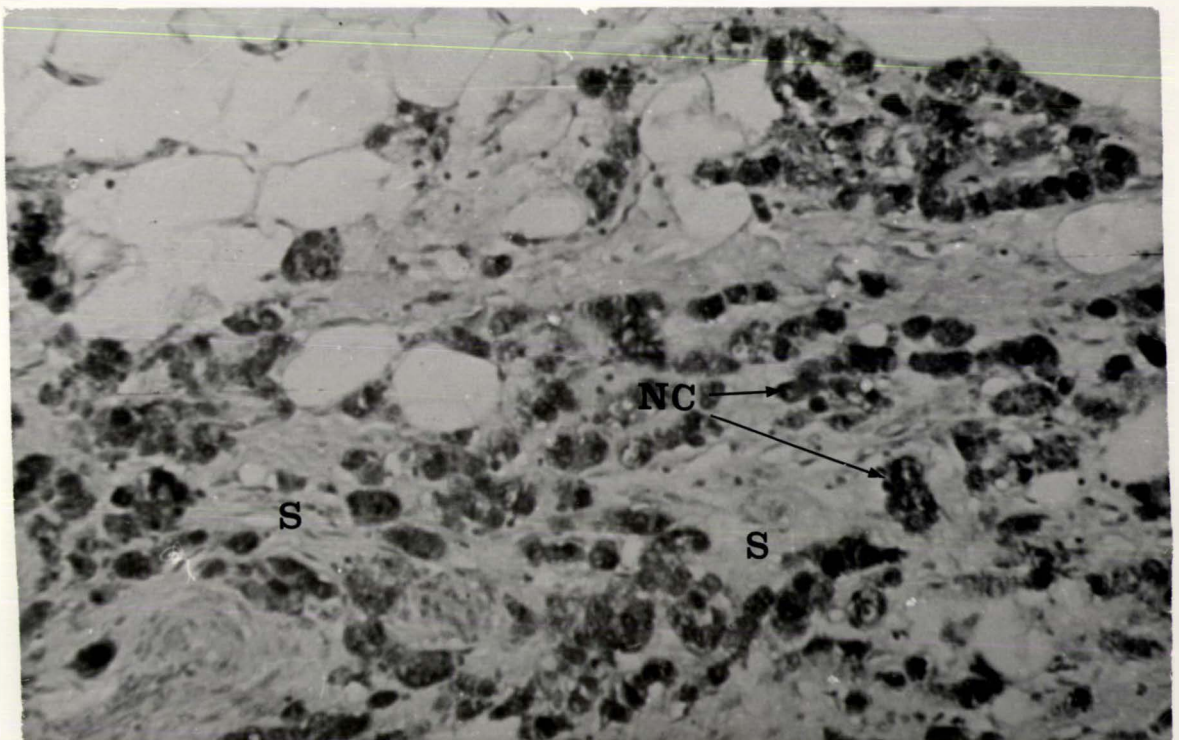
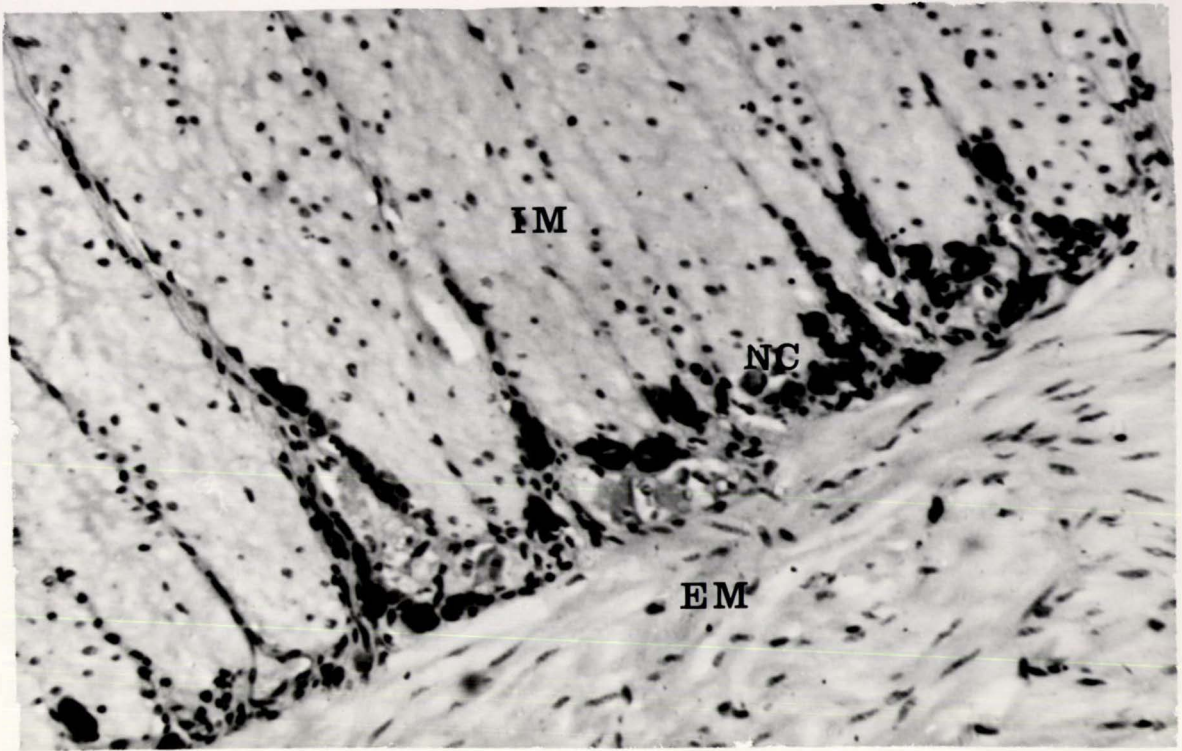
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Figure 32

Aggregations of neoplastic cells (NC) between the internal (IM) and external (EM) muscular layers of the small intestine in the vicinity of a primary small intestinal carcinomatous lesion . H & E. x 150.

Figure 33

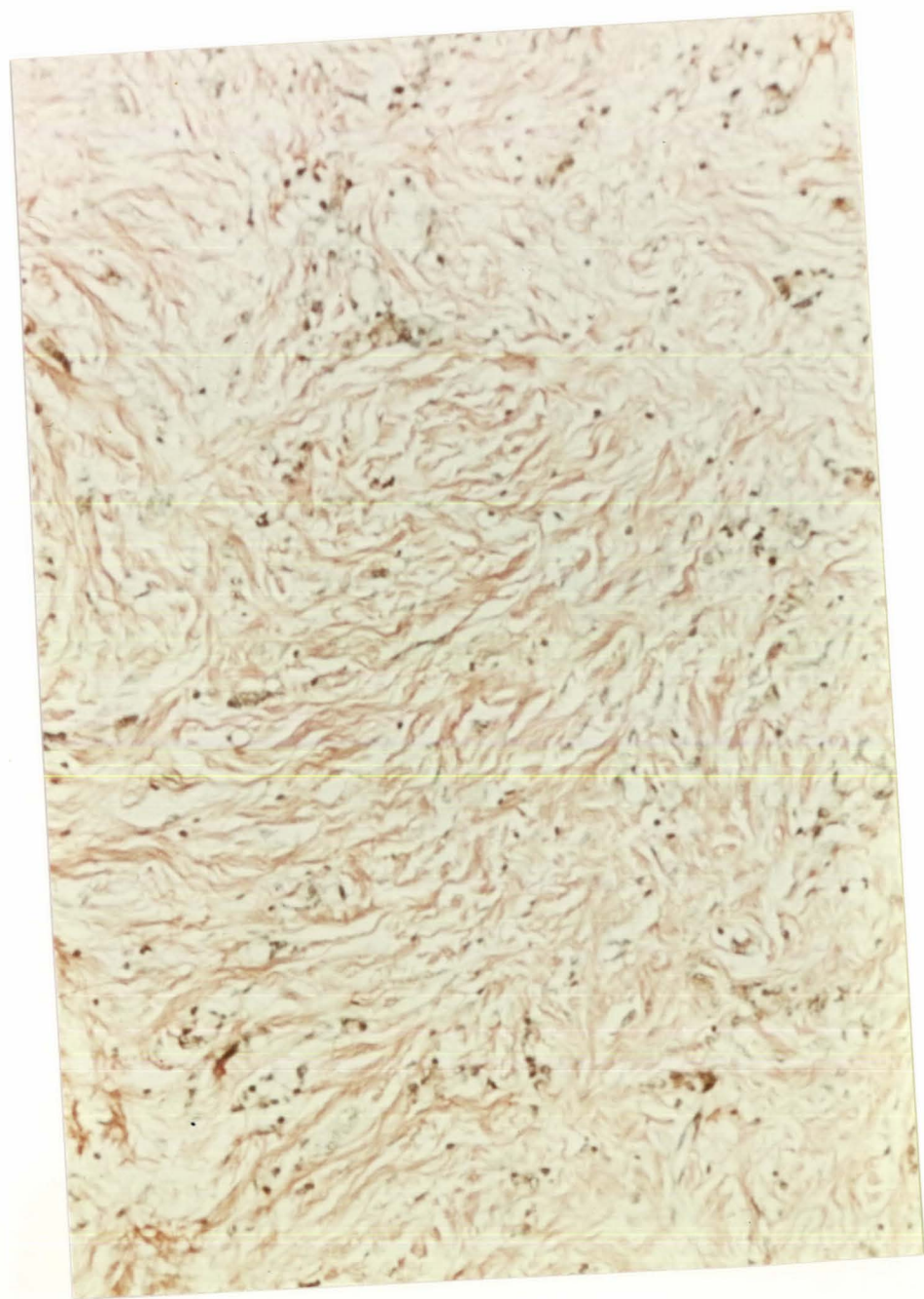
A very small serosal lesion immediately peripheral to the primary lesion of a carcinoma of the small intestine. Neoplastic cells (NC) are relatively numerous in the collagenous stroma (S). H & E. x 150.



following page 31

Figure 34

Serosal lesion of carcinoma of the small intestine showing that collagen makes up a large part of the lesion. van Gieson x 500.



had been almost completely obliterated in many lesions. In other specimens, the submucosa was only slightly thickened and neoplastic cells were mainly seen lining large lymph vessels. Occasional sections showed vessels with a portion of their endothelial lining intact and the remainder replaced by sheets of neoplastic cells. Cords of neoplastic cells were occasionally recognised in small lymph vessels. In one lesion, large clumps of tumour cells were present in lymphatics of the lamina propria (Figure 26) and in another, neoplastic cells formed plaques within lymph vessels immediately beneath the muscularis mucosa (Figure 27). Invasion of the walls of blood vessels (Figures 28 and 29) and of nerves (Figure 30) within the intestinal wall was not uncommon.

Penetration by neoplastic cells through the internal muscular layer took place mainly between muscle bundles (Figure 31), except in a few instances in which a part of the internal muscle layer was extensively infiltrated.

There were commonly aggregates of neoplastic cells between the internal and external muscle layers (Figure 32). Penetration through the outer muscularis occurred in discrete areas. These sites of tumour penetration were seldom observed.

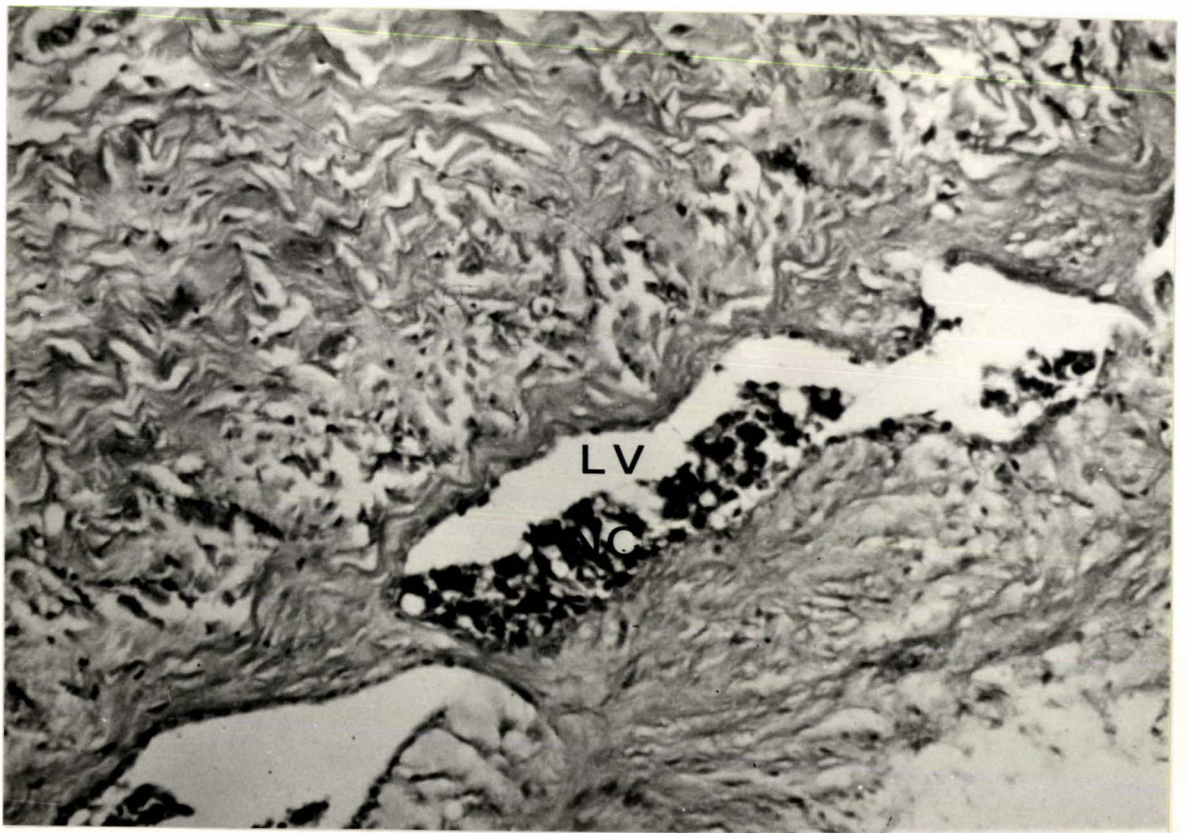
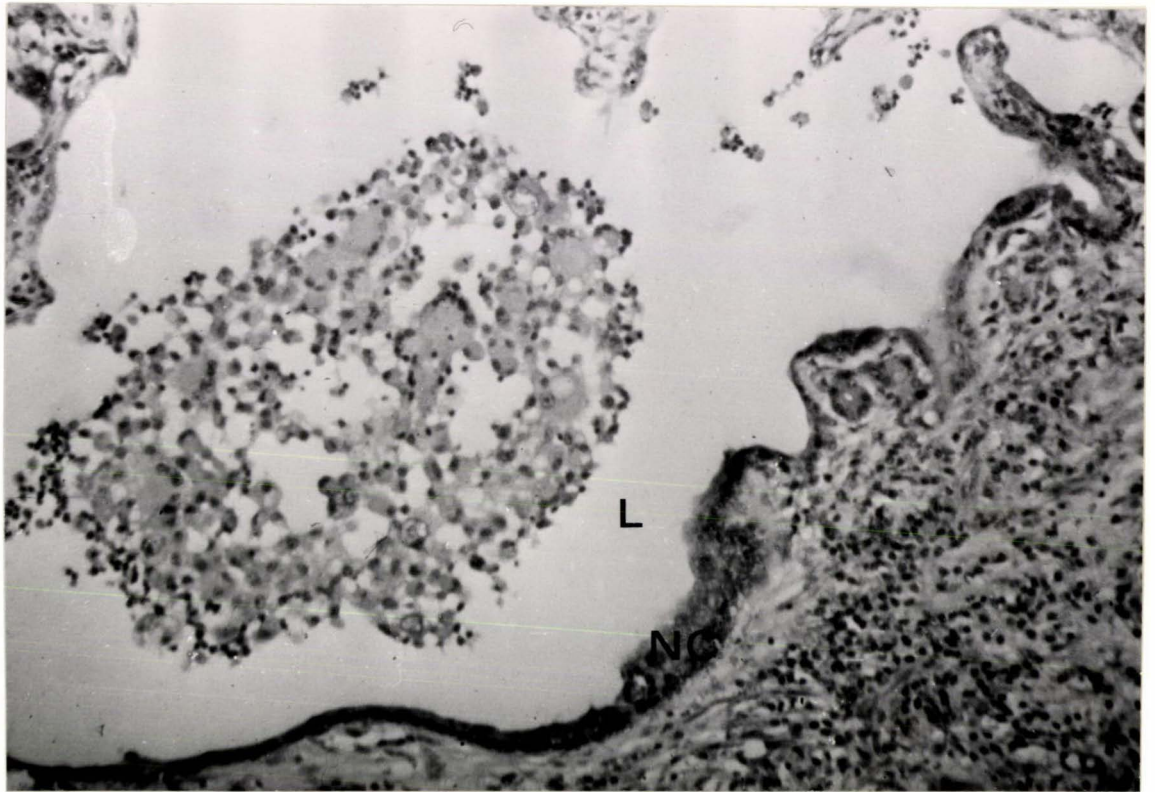
Lesions on the visceral and parietal peritoneum and parietal pleura consisted mainly of collagenous fibrous tissue with a small proportion of neoplastic epithelial cells, mainly situated deep within the lesion. In general, as the size of gross lesions increased, the proportion of neoplastic cells to fibrous stroma decreased, until in many serosal lesions less than 1% of the bulk was made up by carcinomatous cells (Figures 33 and 34). Vascularisation of serosal lesions was slight. Individual small areas of osseous metaplasia were seen in

Figure 35

Lymph vessel appearing as a large acinar structure in a scirrhous serosal lesion. Much of the lining of the lymph vessel has been replaced by neoplastic cells (NC). There is a clump of neoplastic cells in the lumen (L) of the lymph vessel. H & E. x 150

Figure 36

Lymph vessel (LV) in a collagenous lesion on the diaphragm of a sheep with a carcinoma of the small intestine. The lumen of the lymph vessel contains a cord of neoplastic cells (NC). H & E. x 150



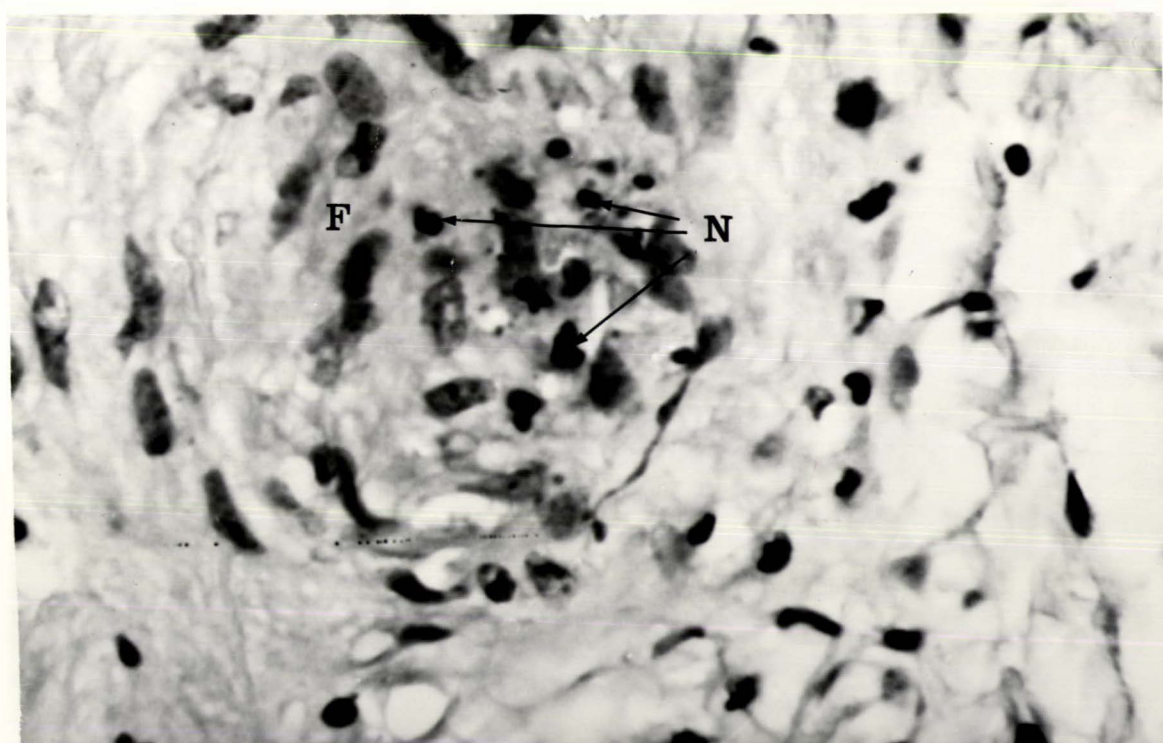
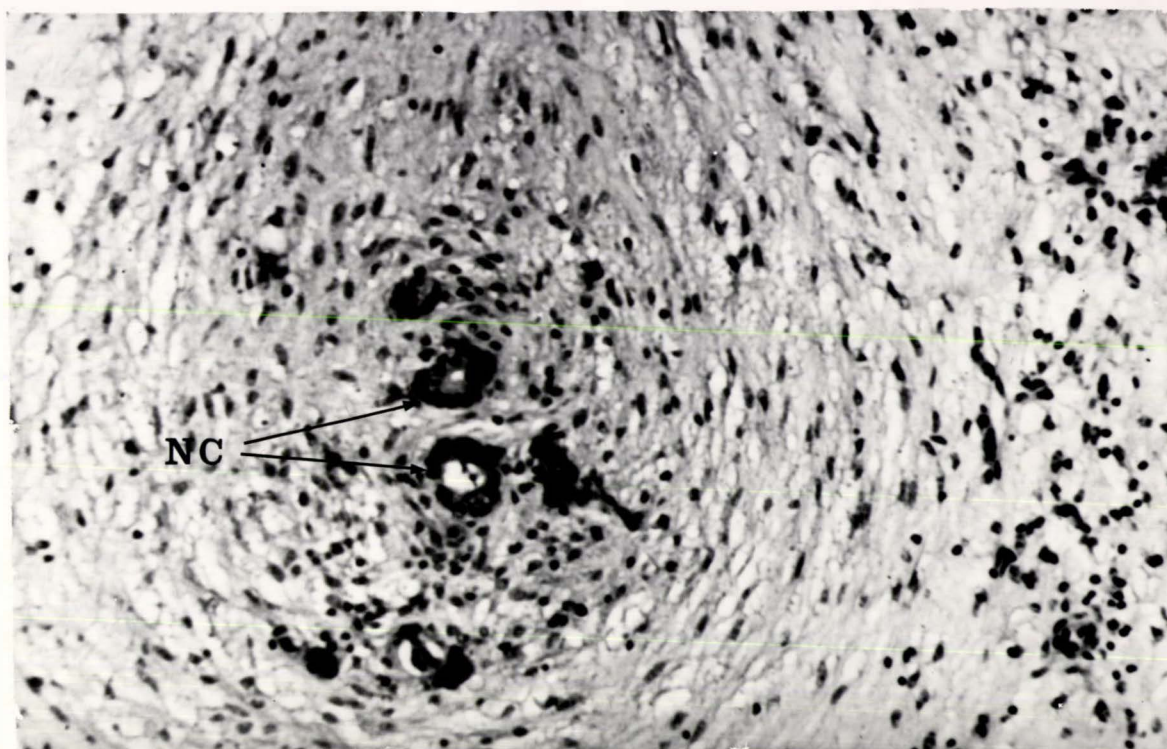
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Figure 37

Acini of neoplastic cells (NC) in a serosal lesion, about which the stroma is orientated in a circular manner. H & E. x 150

Figure 38

Pyknotic nuclear fragments (N) surrounded by immature fibroblasts (F) in a serosal lesion of a small intestinal carcinoma. H & E. x 650.



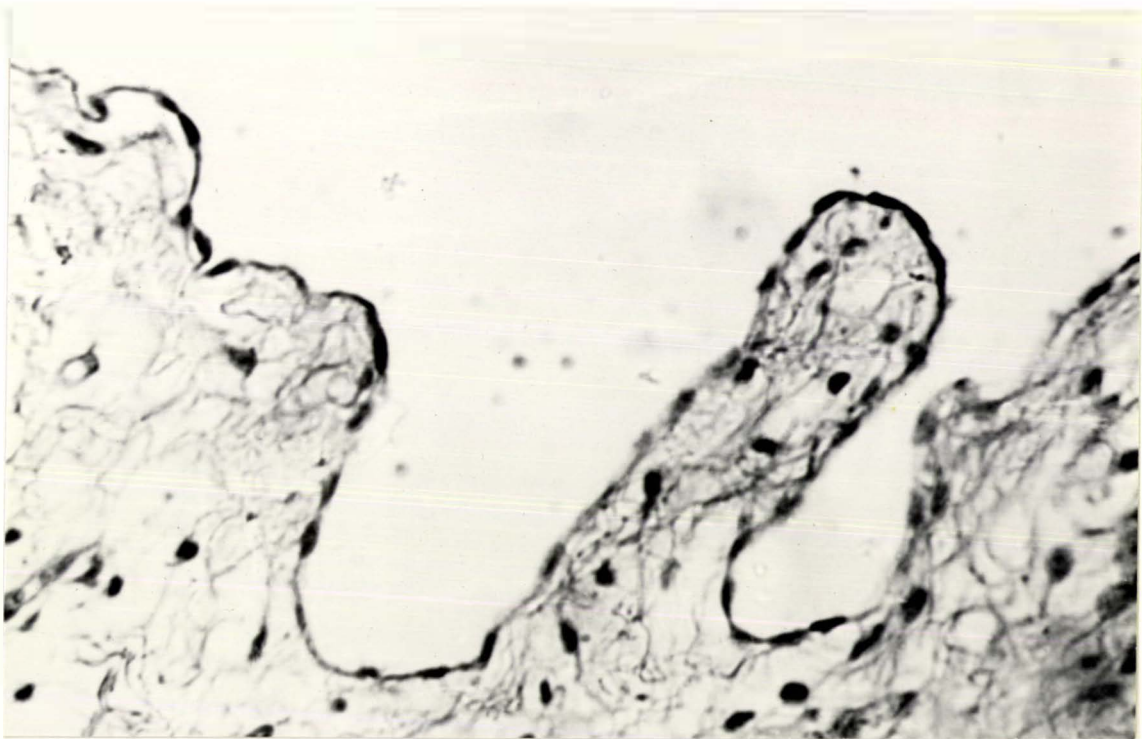
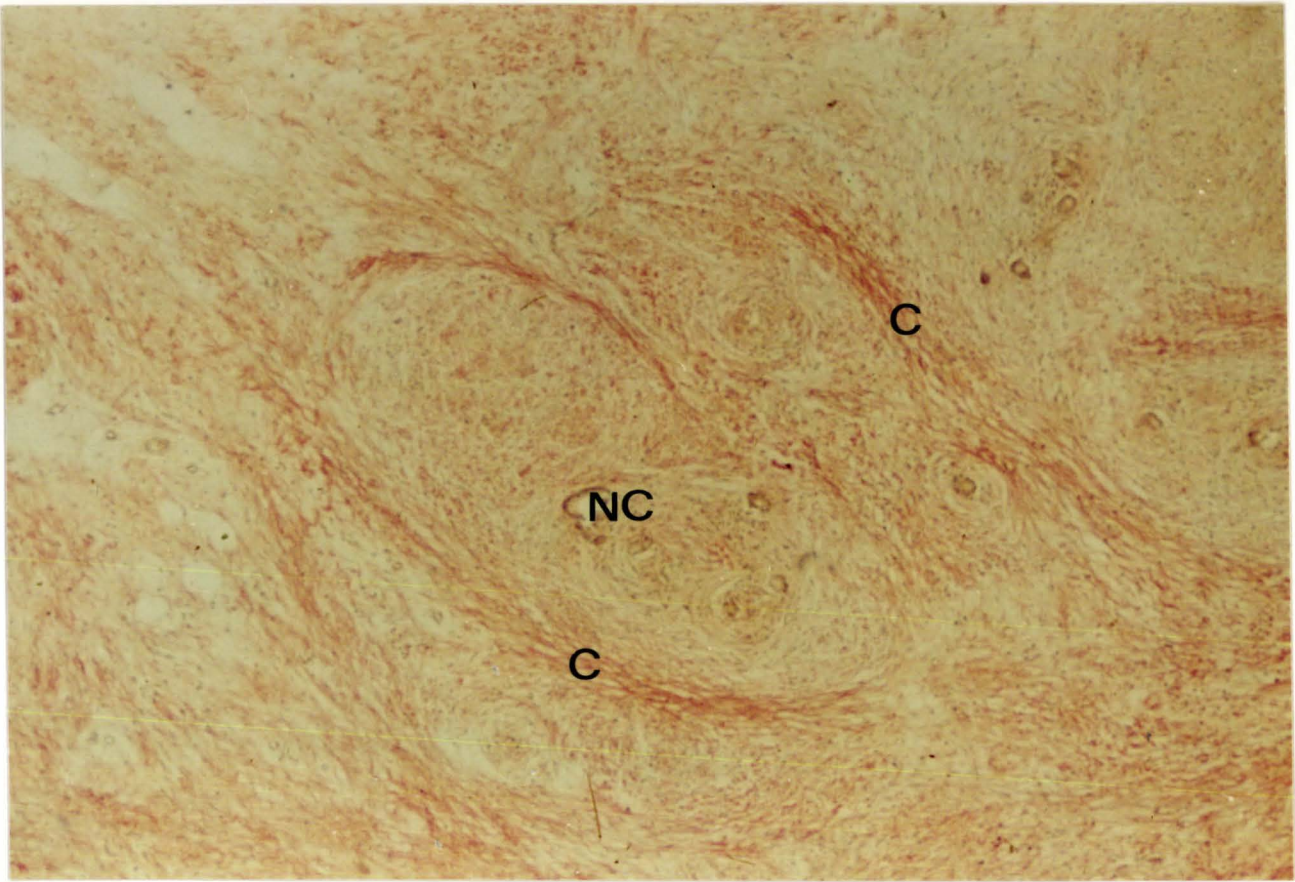
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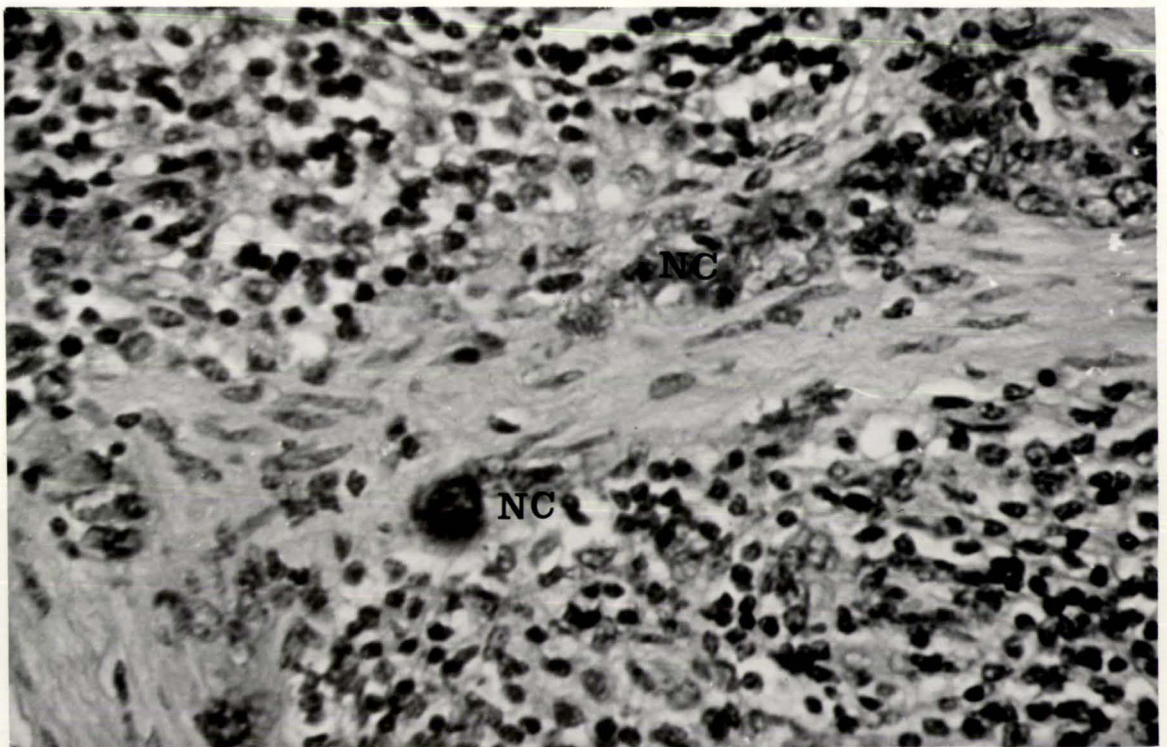
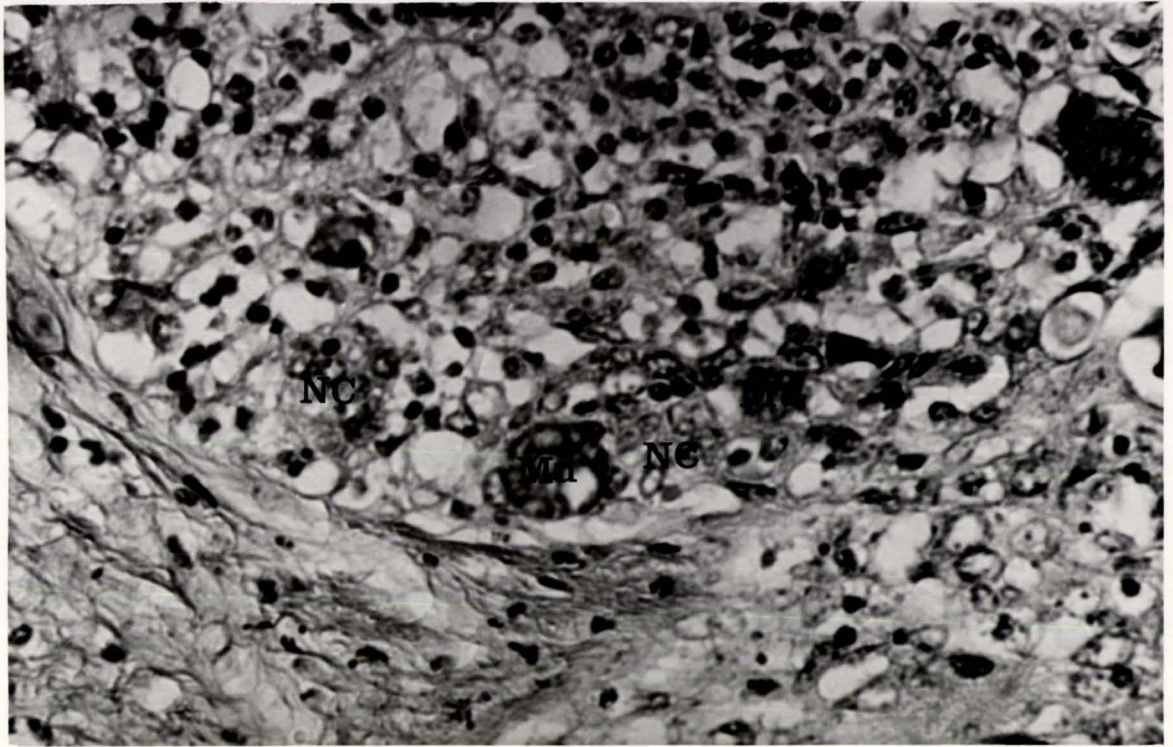
Figure 39

Serosal lesion of a carcinoma of the small intestine with collagen (C) less abundant in the immediate vicinity of neoplastic cells (NC) than elsewhere. van Gieson. x 100.

Figure 40

Loose connective tissue which made up the translucent lesions seen on the proximal portions of the small intestinal wall of some affected sheep. H & E. x 400.





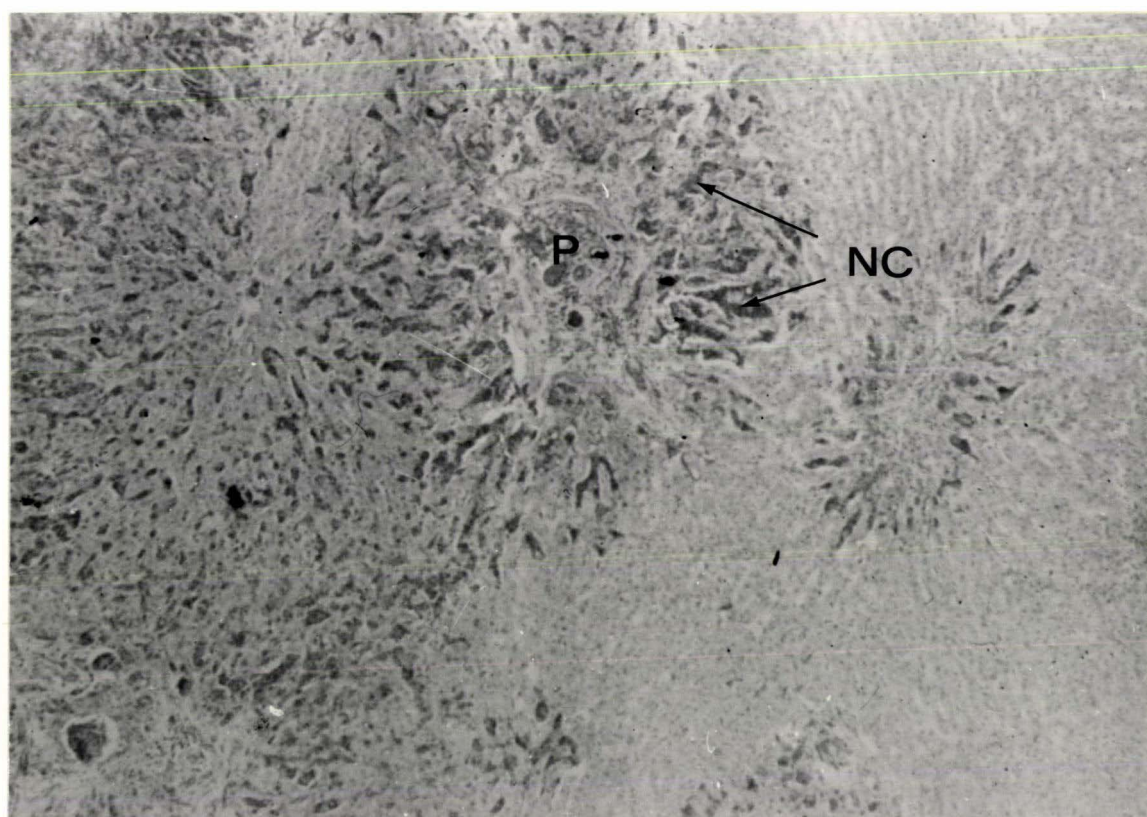
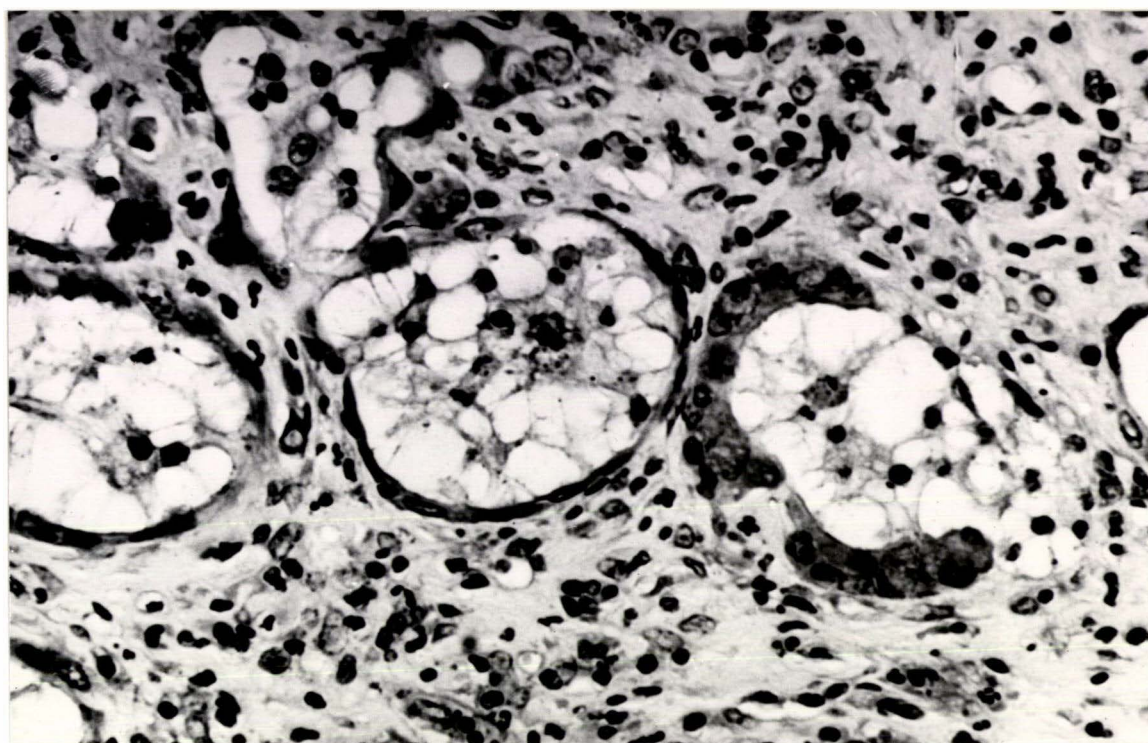
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Figure 43

Acini of neoplastic cells partly replacing the normal tissue of a mesenteric lymph node in a sheep affected by carcinoma of the small intestine. H & E. x 150

Figure 44

Section of a liver containing metastases of a small intestinal carcinoma. Neoplastic cells (NC) are radiating from the portal areas (P) within liver sinusoids. H & E. x 150



serosal lesions in three cases. In some sections of diaphragmatic and parietal pleural lesions the neoplastic cells formed large acinar structures (Figure 35). These were interpreted as lymph vessels lined by neoplastic cells. Groups, and in some cases cords, of neoplastic cells were also seen in lymphatics in diaphragmatic and parietal pleural lesions (Figure 36). In the deeper parts of many serosal lesions, whorls of fibrous tissue surrounded small acini, groups of neoplastic cells or fragments of nuclei of necrotic cells (Figures 37 and 38). In this situation, fibroblasts appeared less mature and with less intercellular collagen than elsewhere (Figure 39).

The pink, translucent lesions noted on the intestinal serosa of some sheep contained no neoplastic cells. These lesions consisted of a loose network of poorly vascularised connective tissue, infiltrated by a few neutrophils and lymphocytes (Figure 40).

The subcapsular, intermediate and medullary sinuses of many affected lymph nodes were extensively invaded by cancerous cells (Figures 41 and 42). In less extensively invaded nodes, the subcapsular and intermediate sinuses only, contained neoplastic cells. Two cases were seen in which the affected mesenteric lymph nodes were largely replaced by neoplastic cells forming large acini (Figure 43).

Metastatic lesions in the liver were centred on portal areas and in some instances the branch of the portal vein contained acini of neoplastic cells. Radiating from the portal area, neoplastic cells infiltrated the sinusoids (Figure 44) and in larger lesions had replaced parenchymal tissue. Neoplastic cells were observed within central veins in some sections. Periportal fibrosis was present in

following page 33

Figure 45

Neoplastic cells, the nuclei of which contain, in one case, two nucleoli (Nu) and in another, an hypertrophied nucleolus (NW).

x 15,000

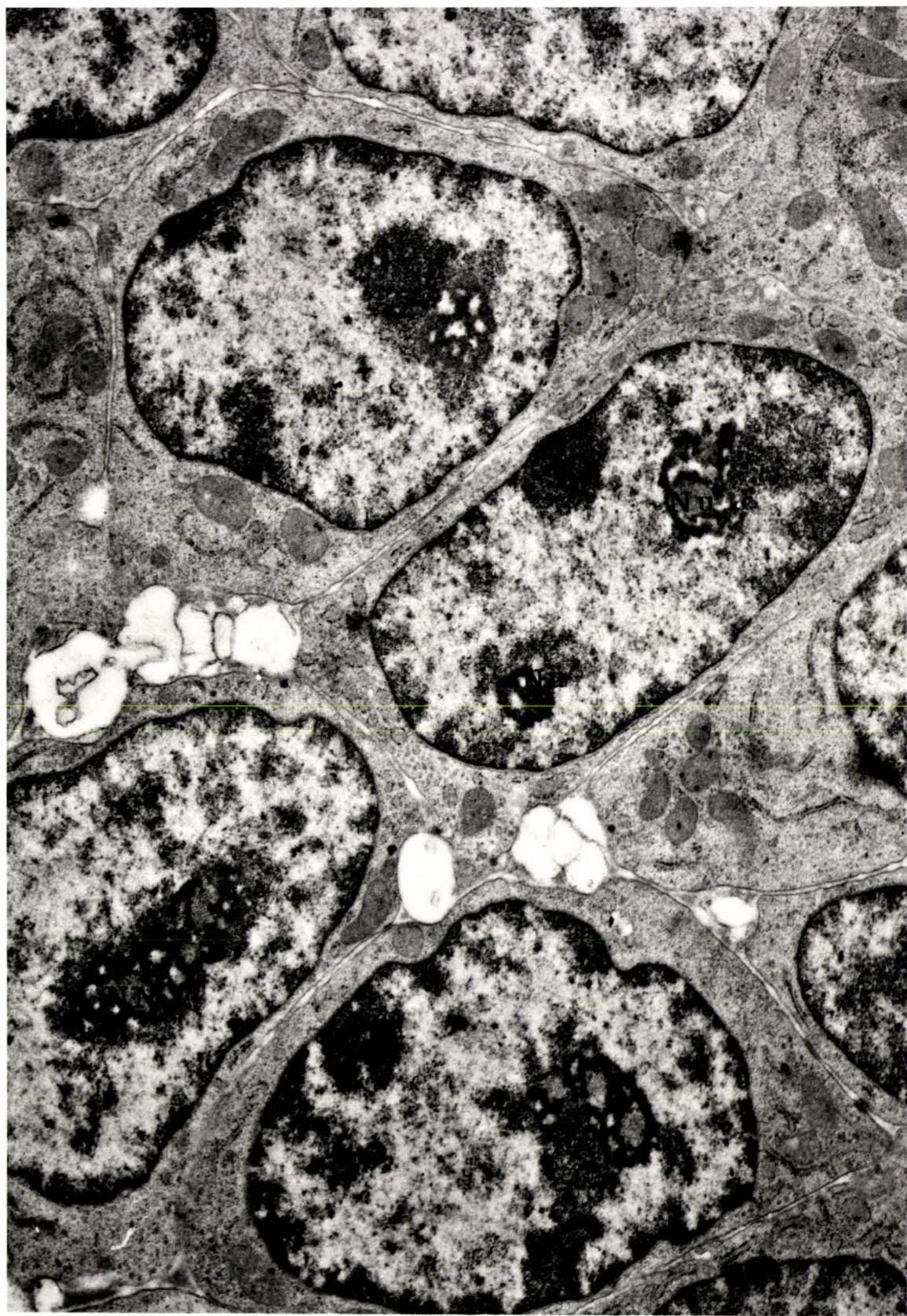
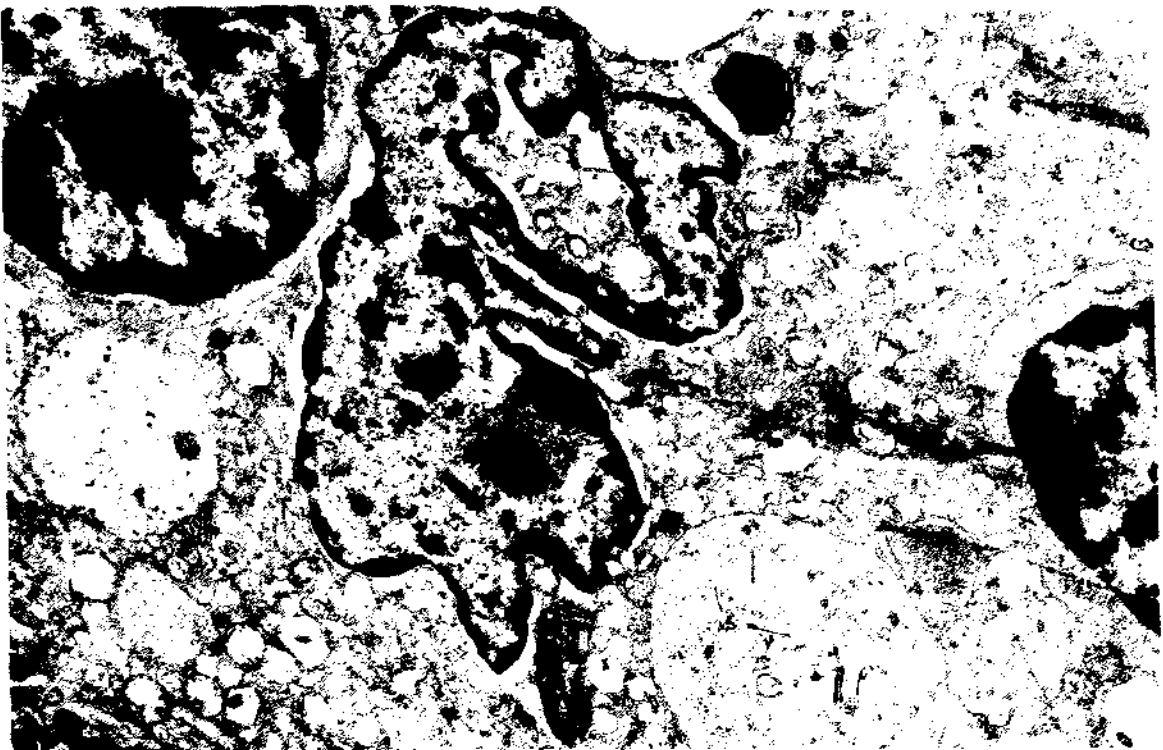
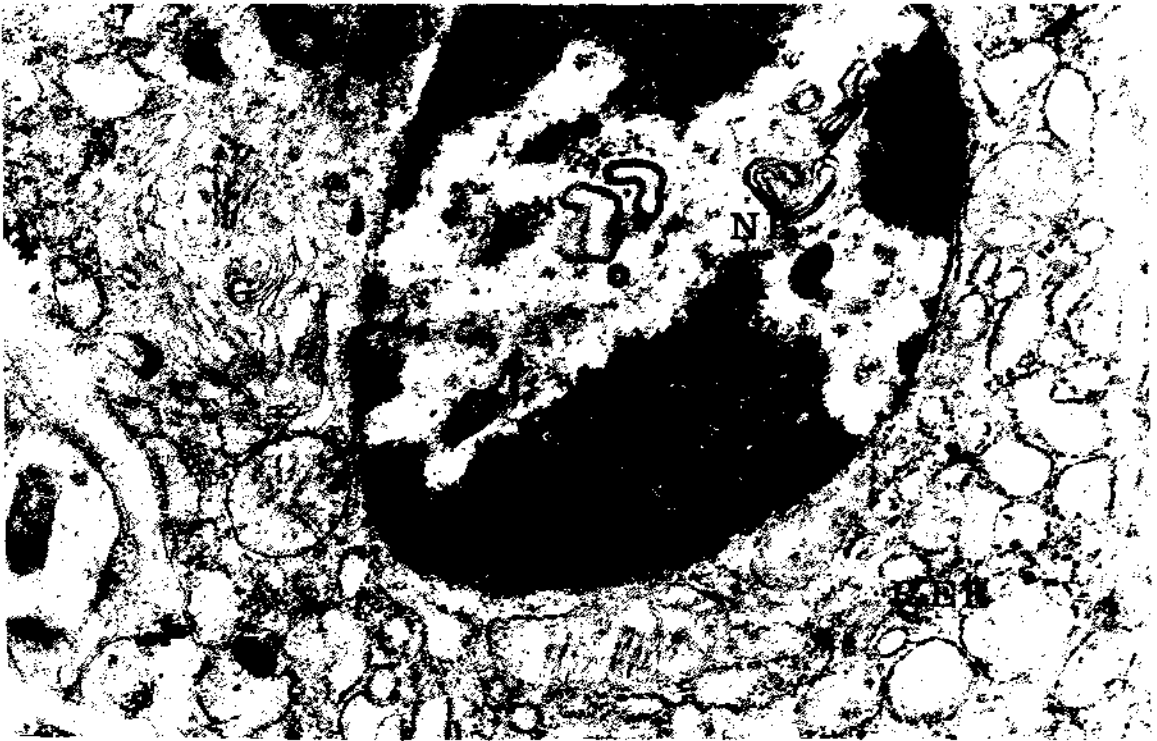


Figure 46

Myelin-like nuclear bodies (NB) in the nucleus of a neoplastic cell. The cytoplasm of the cell contains distended rough endoplasmic reticulum (RER) and a Golgi apparatus (G). x 22,000

Figure 47

The nucleus of a neoplastic cell, the nuclear membrane of which is deeply folded. x 10,000



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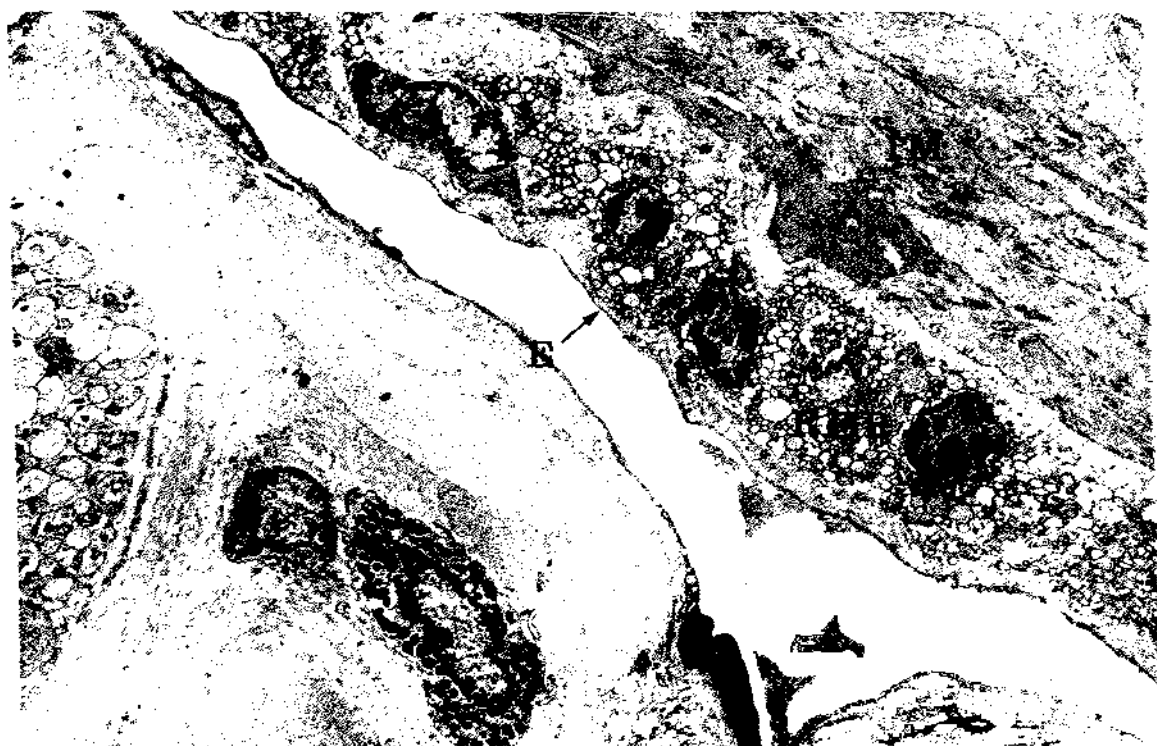
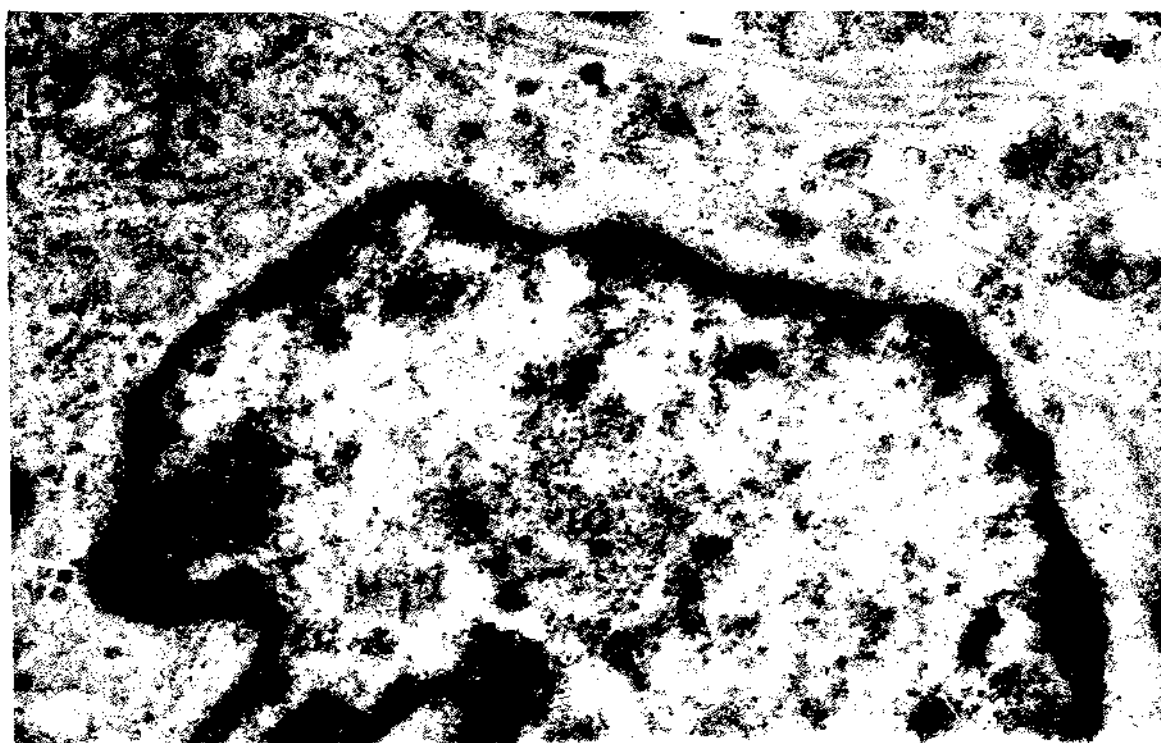
Figure 48

Interchromatin granules (IG) in the nucleus of a neoplastic
cell. x 25,000

Figure 49

Neoplastic cells beneath the endothelium (E) of a lymph vessel
in the internal muscularis (IM) of the intestinal wall. The rough
endoplasmic reticulum (RER) of the neoplastic cells is distended.

x 3,000



hepatic metastatic lesions.

Extensive fibrous tissue formation, as seen in serosal lesions, was not a dominant feature of lesions of either the mucosal, submucosal or muscular layers of the small intestine, or in lesions of the liver or lymph nodes.

Ultrastructure of Small Intestinal Carcinomas

Examination of tumours by means of the electron microscope revealed considerable variations in dominant cell type between portions of tumours. In some sections, the predominant neoplastic cells resembled the columnar epithelial absorptive cells more closely than they did other cell types of the intestine. They had large nuclei in which the chromatin tended to be aggregated peripherally. The nucleoli, of which there were sometimes two per nucleus, were frequently hypertrophied (Figure 45). Intranuclear myelin-like figures were occasionally seen (Figure 46), and pronounced folding of the nuclear membrane was sometimes present (Figure 47). Aggregations of electron dense particles approximately 200 Å in diameter were distinguishable from chromatin granules in some nuclei (Figure 48). The cytoplasm of this cell type varied considerably in its content of rough endoplasmic reticulum, and in some cells where it was abundant it was distended (Figures 46 and 49). Free ribosomes were never plentiful, and mitochondria were generally rather few in number but large in size. Golgi apparatuses were seen in some cells (Figure 46).

In other blocks, the most common neoplastic cell was one more closely resembling intestinal goblet cells. The predominant feature

following page 34

Figure 50

Intracytoplasmic mucous droplets (Mu) of varying size and electron density in a group of neoplastic cells. Collagen fibres (C) are in close proximity to the neoplastic cells. x 10,000

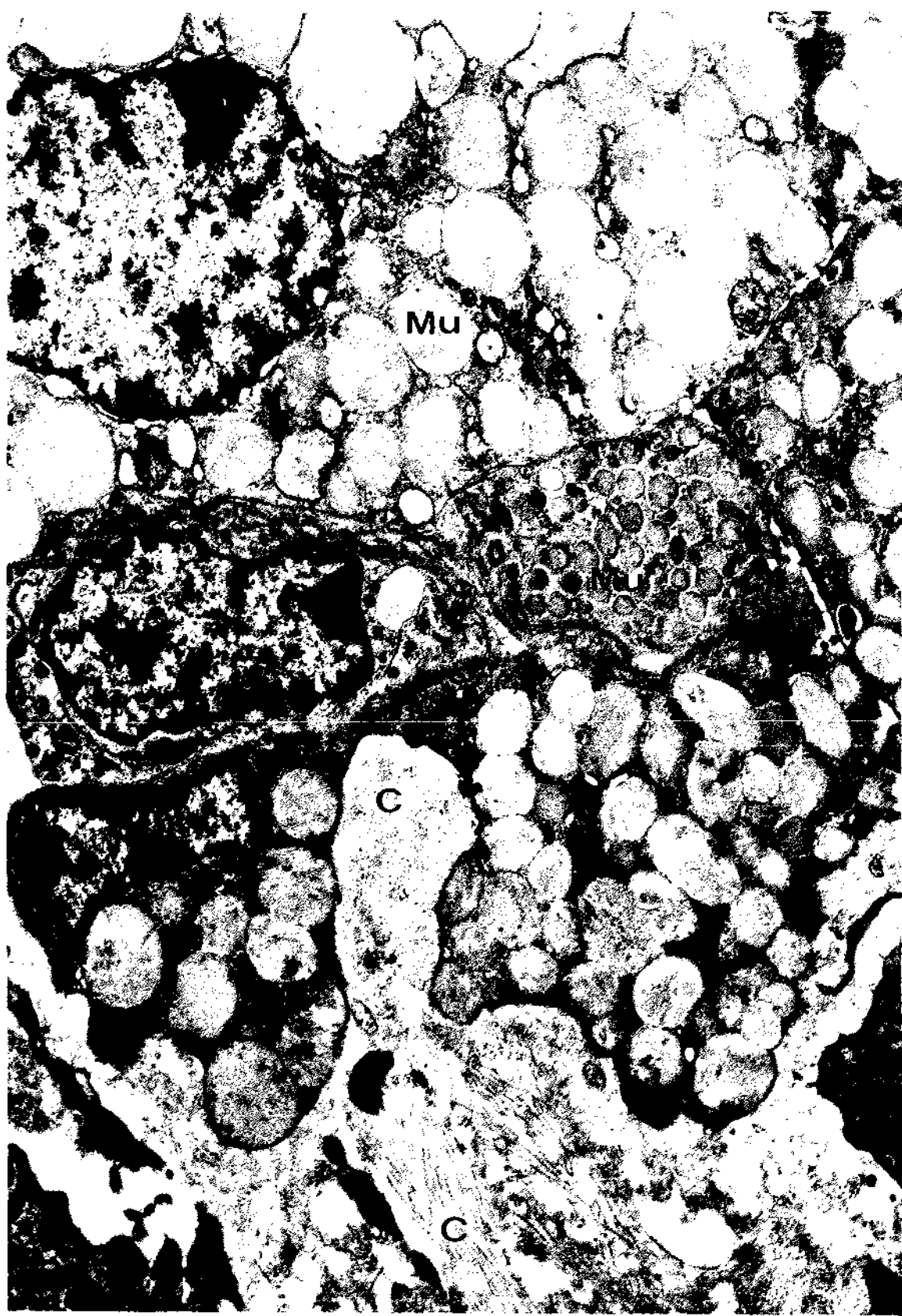
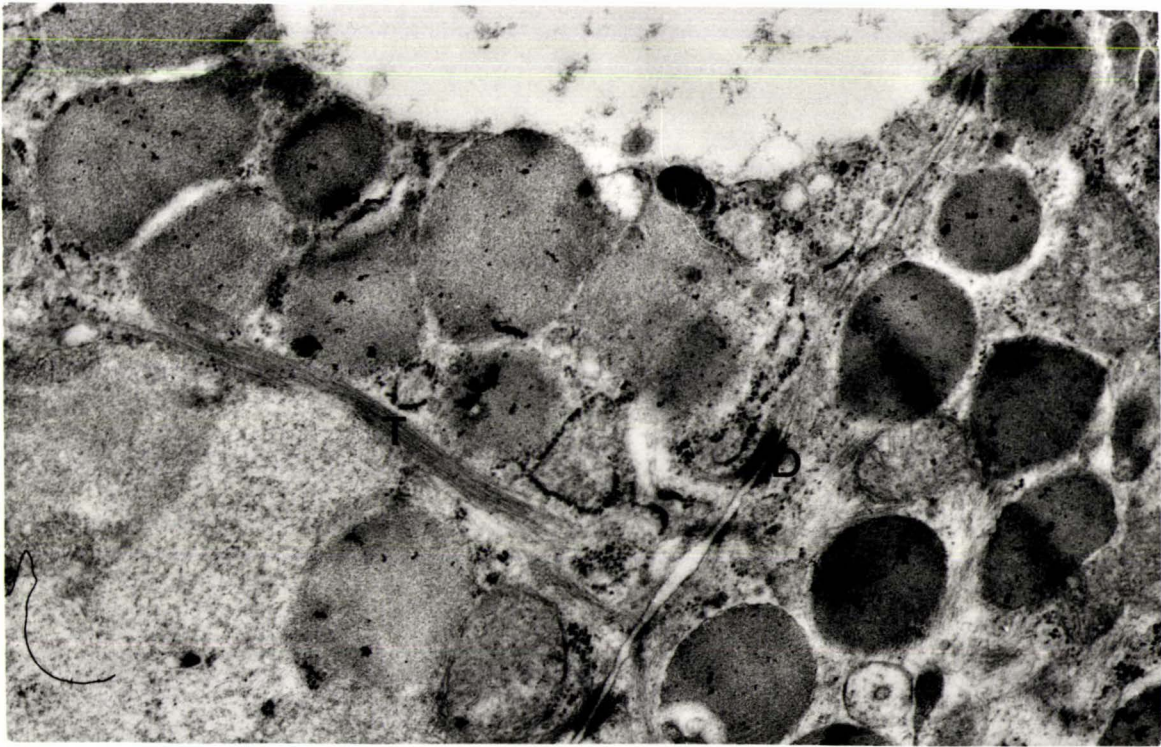
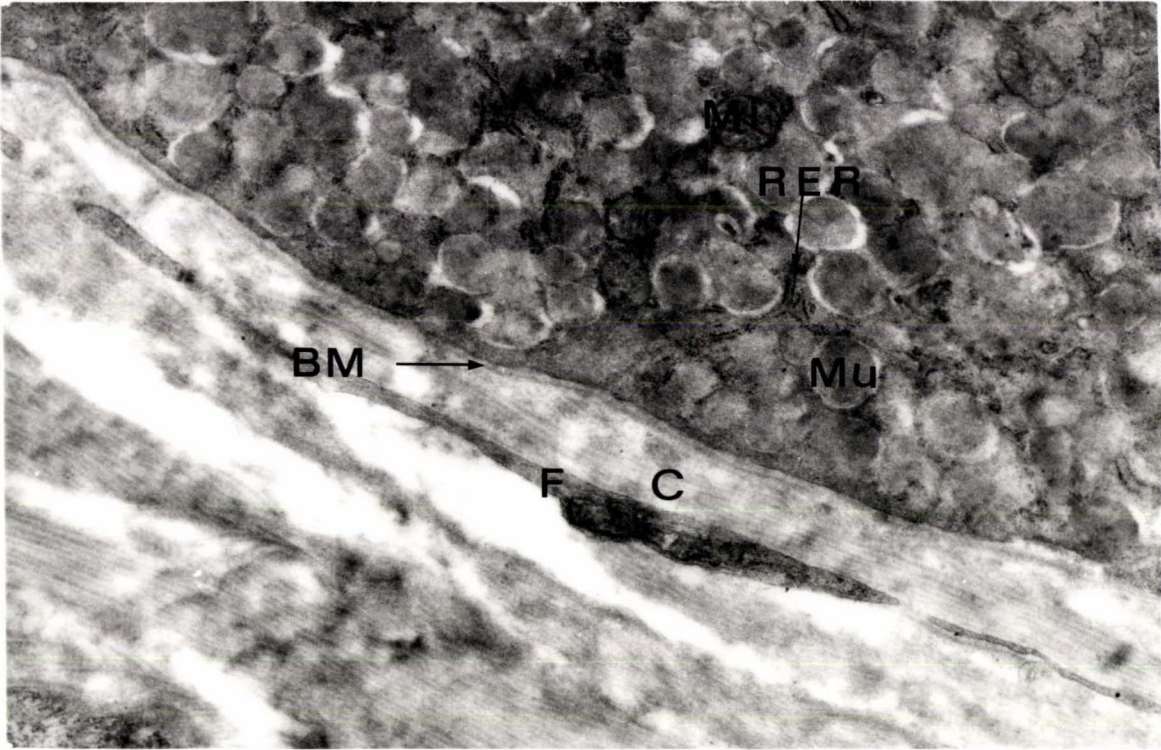


Figure 51

The border of a neoplastic cell containing mucous droplets (Mu) between which there is rough endoplasmic reticulum (RER) and mitochondria (Mi). The surface of the cell is invested with a basement membrane (BM) immediately adjacent to which are collagen fibres (C). A fibrocyte (F) is aligned parallel to the cell surface and only slightly separated from it. x 15,000

Figure 52

Portions of the cytoplasm of two neoplastic cells, one of which contains tonofibrils (T), and between which there are desmosomes (D). x 25,000



following page 34

Figure 53

A group of neoplastic cells, the borders of which show deep folding and interdigitation. Desmosomes (D) are present between two cells. The basement membrane (BM) surrounding the cells is hypertrophied in one area (HBM) x 18,000



following page 34

Figure 54

A neoplastic cell, containing a large intracytoplasmic vacuole which is lined by microvilli (MV) and contains an amorphous material (Mu). The boundaries of this, and the adjoining, cell are deeply folded and interdigitated. x 12,500



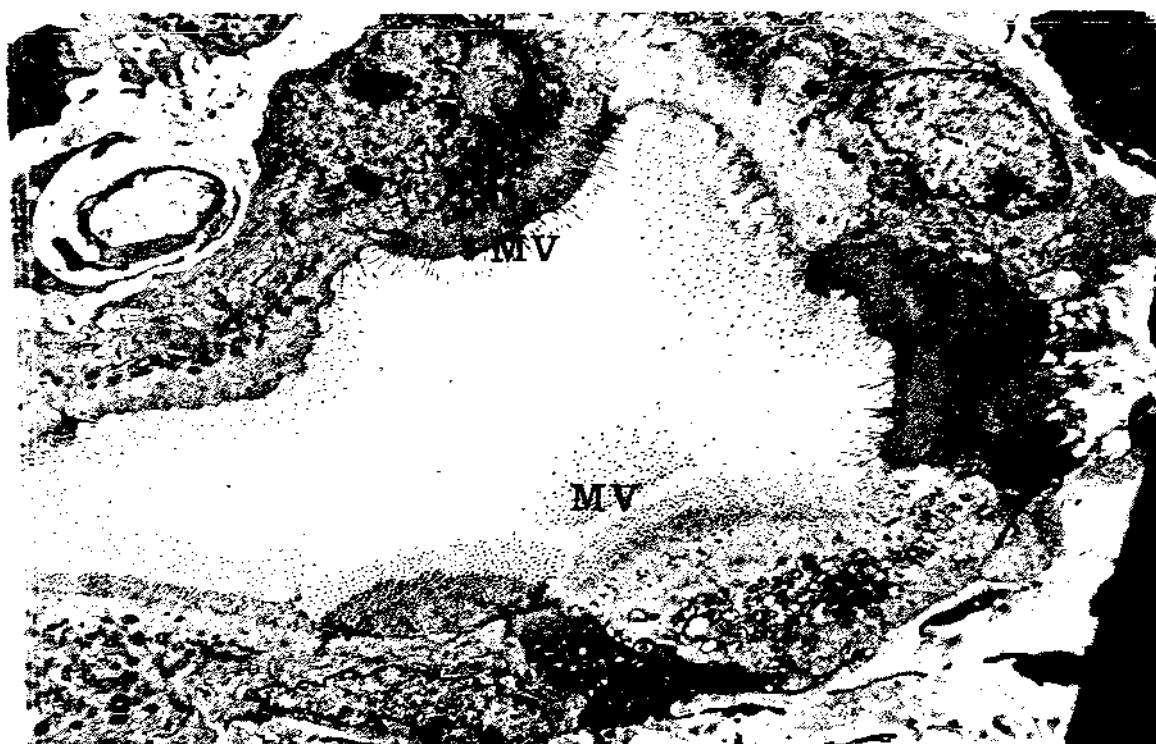
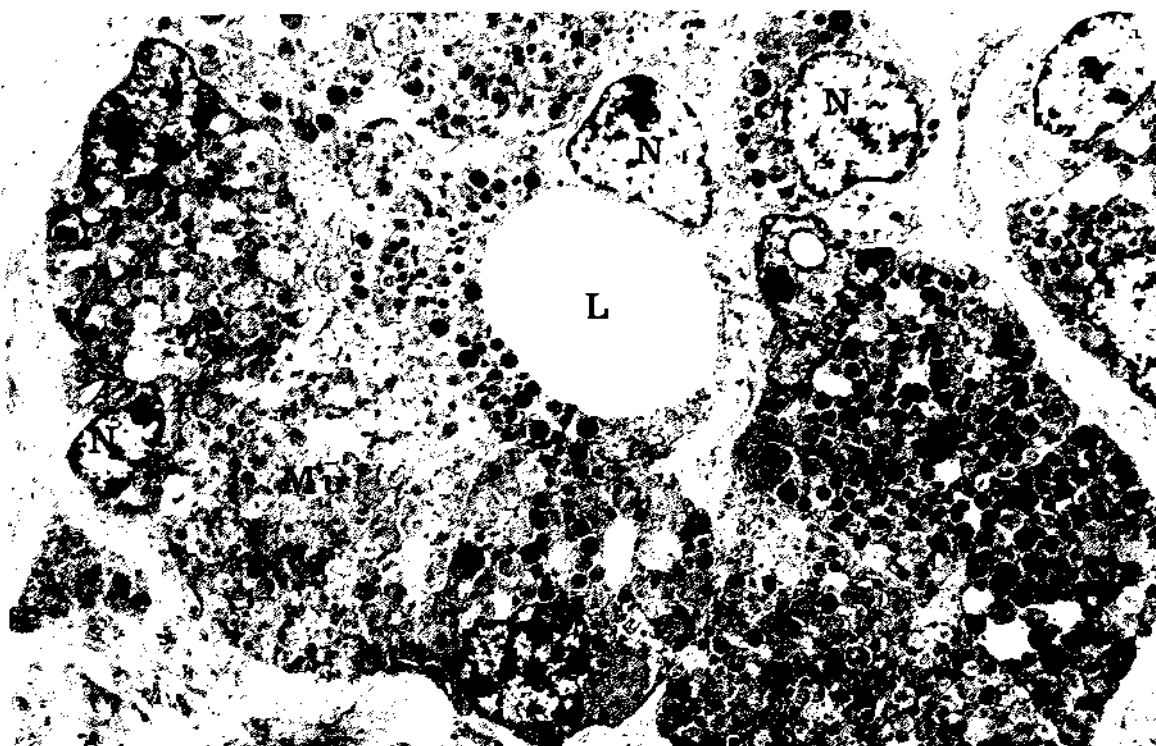
Figure 55

An acinus of cells containing mucus (Mu). Nuclei (N) show variable polarity with respect to the lumen of the acinus (L).

x 3,500

Figure 56

An acinus of non mucus-producing cells. The luminal surfaces of all cells in this section give rise to large numbers of microvilli (MW). The areas of cytoplasm immediately beneath the microvilli are devoid of organelles. x 4,000



following page 34

Figure 57

An acinus of neoplastic cells, some of which contain mucus (Mu) and some of which do not. x 4,000

Figure 58

The luminal border of cells in an acinus, showing microvillus (MV) formation and an area of cytoplasm beneath the microvilli which is devoid of organelles. x 9,000



of these cells was mucus in the form of large numbers of spherical, homogeneous, intracytoplasmic inclusions of variable size and electron density (Figure 50). Golgi apparatuses were present in these cells and rough endoplasmic reticulum and mitochondria occupied much of the remaining cytoplasm (Figure 51). Tonofibrils were not common but were seen occasionally (Figure 52). Rarely, there was aggregation of mucous droplets with disruption of the cell membrane in an apparent attempt to secrete mucus into the intercellular space. The nucleoli of mucus-containing cells were similar to those of non-mucus-producing neoplastic cells.

Desmosomes of neoplastic cells were normal, but varied in number between portions of tumours. They were considered more common between mucus-containing than between non-mucus-containing cells (Figures 52 and 53). Between some neoplastic cells there was more pronounced folding and interdigitation of cell membranes (Figures 53 and 54) than was present between crypt epithelial cells in the normal tissues examined.

Acinus formation was common, by both mucus-producing and non-mucus-producing neoplastic cells (Figures 55 and 56). Both cell types were present in some acini (Figure 57). The luminal borders of cells forming acini were sometimes lined by microvilli (Figures 56 and 58). Where microvilli were present, they showed more variation in length and numbers than microvilli on normal crypt epithelial cells. Ultrastructurally, they resembled normal microvilli, being covered by a cell membrane and having a number of filaments running along their length. These central filaments penetrated to variable distances beneath the cell surface but the formation of distinct terminal webs

following page 35

Figure 59

The border of a neoplastic cell, on which there is a discontinuous basement membrane (BM) with cytoplasm (C) protruding through the gap. Arrows indicate the positions at which the basement membrane ends (N = nucleus) x 48,000.

Figure 60

A large, intracytoplasmic vacuole (V) with no microvilli (N = nucleus, arrows indicate the cell membrane). x 12,000

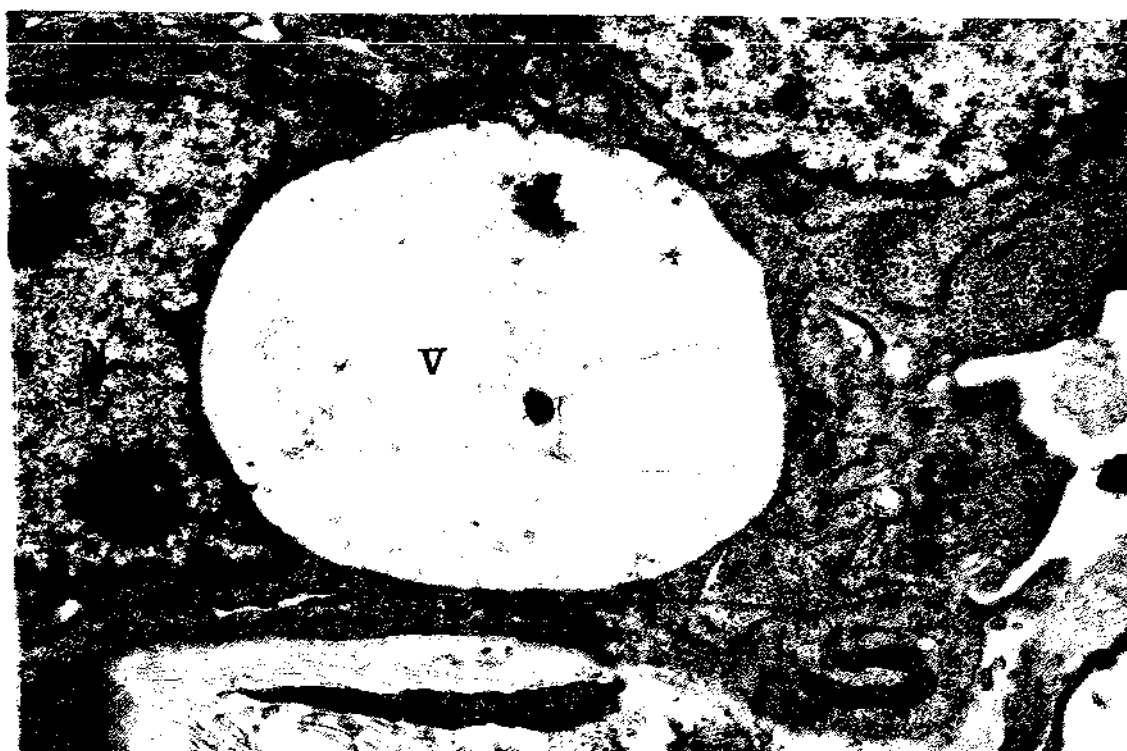
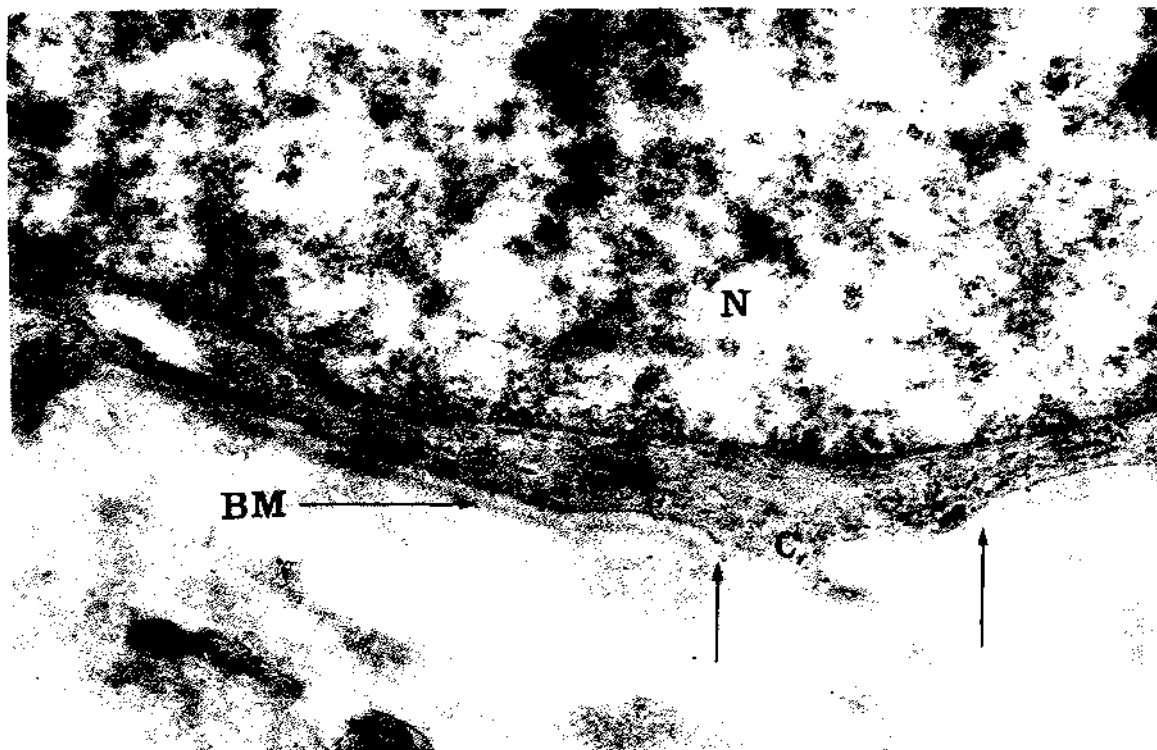


Figure 61

An intracytoplasmic vacuole in a neoplastic cell. The vacuole is lined by microvilli (MW) which have filaments (F) running along their length and into the underlying cytoplasm. The vacuole contains a finely granular material (Mu) x 16,000

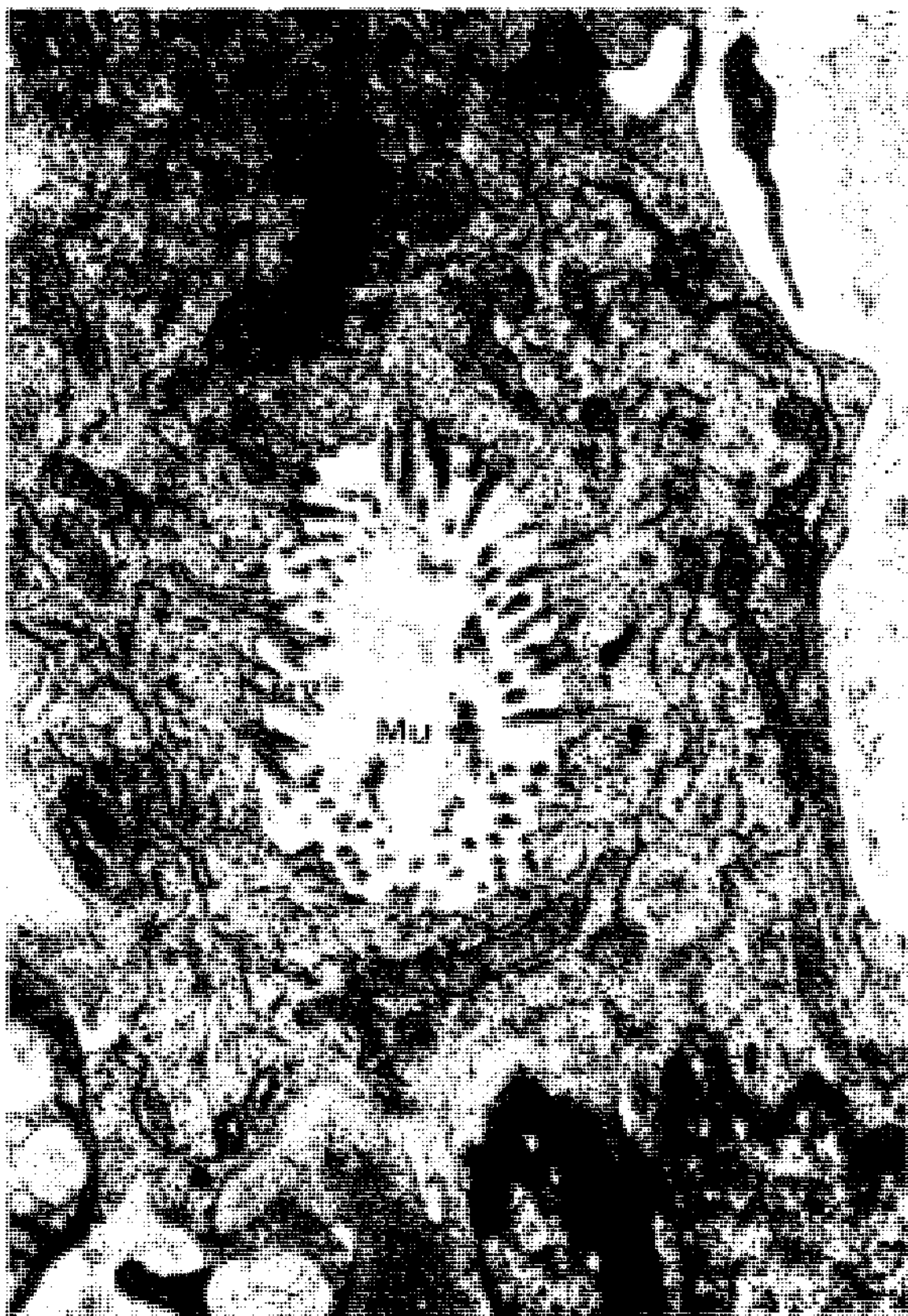
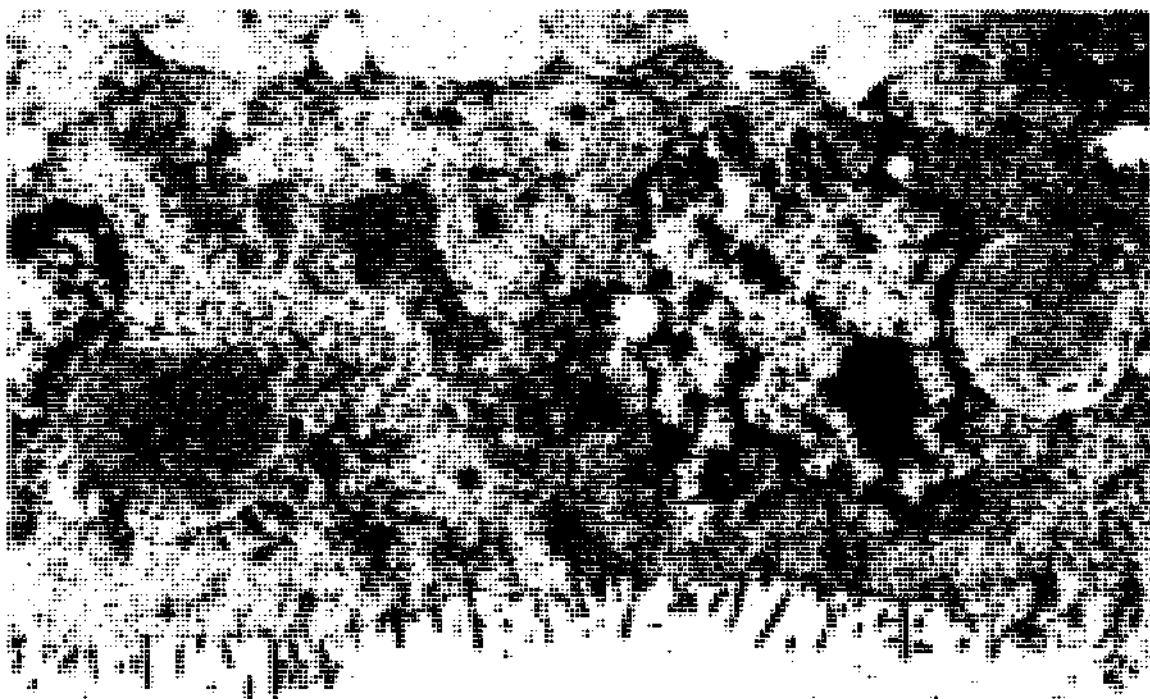


Figure 62

Cuboidal epithelial cells lining the intact luminal surface of a small intestinal carcinoma. There are short microvilli (MV) on the luminal surface and two of the cells contain single droplets of mucus (Mu). x 9,000

Figure 63

A portion of a serosal lesion in a sheep with a carcinoma of the small intestine. The lesion consists largely of fibrocytes (F) and collagen fibres (c). x 3,000



or terminal bars was not observed. Beneath cell surfaces on which microvilli were present, there was frequently a considerable depth of cytoplasm within which there were few or no intracytoplasmic organelles (Figure 58).

Surrounding acini and groups of cells, especially groups of mucus-producing cells, there was often a basement membrane similar in dimensions and electron density to that beneath normal crypt epithelium (Figure 51). Occasionally, this basement membrane was discontinuous and there were cytoplasmic protrusions extending through the gaps (Figure 59). Hypertrophy of basement membranes (Figure 53) was only occasionally seen.

In some sections, a variable proportion of cells contained large intracytoplasmic vacuoles of varying sizes, lined by microvilli and containing amorphous or finely granular material (Figures 54 and 61). These vacuolated cells did not contain intracytoplasmic mucous droplets. The microvilli lining the vacuoles were variable in length and numbers, but were otherwise similar in structure to those lining intestinal crypts. The underlying cytoplasm sometimes contained intracytoplasmic organelles (Figure 61) and so differed from the cytoplasm beneath the brush border of normal or neoplastic cells. Very occasionally, similar large vacuoles were seen in which there were no microvilli (Figure 60).

The luminal surface of some small intestinal carcinomas was lined by a layer of cuboidal cells. These had fewer and shorter microvilli than normal intestinal epithelial cells or neoplastic cells forming acini. A small proportion contained a single large droplet of mucus (Figure 62).

following page 36

Figure 64

A portion of a serosal lesion in a sheep with a carcinoma of the small intestine. The lesion consists, largely, of fibrocytes (F) and collagen fibres (C). (N = nucleus; RER = rough endoplasmic reticulum) x 14,000



Neoplastic cells were occasionally noted within small lymphatics and capillaries. In one section, invasion by tumour through the internal muscularis was seen to be along the outside of a lymph vessel (Figure 49).

Electron microscopic observations of the stroma of mucosal lesions, and of the serosal scirrhous lesions (Figures 63 and 64), were in accord with those made with the light microscope. Of note, was the close proximity of collagen fibres to neoplastic cells (Figures 50 and 51). Fibrocytes were commonly orientated parallel to the cell surface and only slightly separated from it (Figures 51 and 57).

DISCUSSION

The degree of uniformity in the gross pathology of small intestinal carcinomas examined in the course of this investigation was remarkable. Every lesion conforming to the gross description given earlier in this chapter, which was examined histologically, was confirmed as an intestinal carcinoma. In no case was a carcinoma diagnosed from histopathological examination of a variety of lesions not conforming to that description (see page 61). Differences noted between lesions pertained mainly to the exact location and degree of development and dissemination of lesions.

Histologically, all intestinal neoplasms examined were also very similar, both in the appearance of neoplastic cells and in the degree of stroma formation. The extent to which neoplastic cells were differentiated toward acinus formation and mucus production varied

almost as much between parts of lesions as between neoplasms. In all tumours, some neoplastic cells produced mucus while other cells did not. Similarly, the ultrastructure of neoplastic cells in all seven lesions examined was similar. Within each lesion, neoplastic cells existed in two main forms. Some resembled goblet cells, while others more closely resembled columnar absorptive cells. From these gross, histological and ultrastructural similarities, it was concluded that these neoplasms represented a single pathological entity.

The appearance of two predominant cell types in each neoplasm is not considered indicative of a mixed neoplasm. Rather, it is believed this reflected the origin of these tumours from a relatively undifferentiated cell type in the crypts of Lieberkuhn, which having undergone neoplastic transformation, was still capable of specialisation of function. It has been suggested that goblet cells may originate from independent embryological elements in the intestinal crypts (Cowdry, 1963). However, differences between "resting goblet cells" and columnar cells are small, if they exist at all, and it appears to be more generally accepted that goblet cells originate by differentiation of columnar absorptive cells (Palay, 1958; Freeman, 1962). Goblet cells have rarely, if ever, been observed in mitosis, and it is not common to find goblet cells in the small intestine adjacent to one another, as might be expected if they originated from independent elements (Arey, 1968). Differentiation of a proportion of neoplastic cells toward mucus production appears a more likely source of mucus producing cells than does the independent neoplastic transformation of two cell types in each of the intestinal neoplasms examined.

The observed cytological abnormalities of peripheral aggregation of nuclear chromatin, nucleolar hypertrophy, multiple nucleoli, increased folding of the nuclear surface, variations in desmosome numbers, and hypertrophy of basement membranes in neoplasms examined in this study have been described previously in cells of other neoplasms (Haguenau, 1969). The myelin-type intranuclear inclusions observed in these tumours correspond to the "nuclear bodies" previously reported as more predominant in neoplastic than in normal cell nuclei (Weber, Whipp, Usenik and Frommes, 1964; Krishan, Uzman and Hedley-Whyte 1967; Haguenau, 1969). The electron dense particles, 200 Å in diameter, observed aggregated in nuclei, correspond to the "interchromatin granules" first named by Swift (1959) and subsequently shown to occur in a wide range of neoplasms (Haguenau, 1960, 1969). All these features have previously been described in neoplastic cells and many have been observed, though less conspicuously, in normal cells. Haguenau (1969) considered these, and other observed cellular abnormalities of neoplasms, reflected alterations (generally increases) in cellular metabolism accompanying neoplastic transformation.

Intracytoplasmic, microvillus-lined, mucus-containing vacuoles similar to those seen in intestinal carcinomas of sheep, have previously been reported only from human stomach carcinomas. With the aid of serial sections, Kondo, Tamura and Taniguchi (1970) demonstrated that these structures were intracellular sacs containing mucus, and were therefore most probably unrelated to parietal cell canaliculi as had previously been suggested by Seki (1962) and Cota (1965). The name "intracellular microcyst" was suggested for these structures by Kondo et al. (1970). Microcysts

observed in carcinomas of the intestine of sheep appear to be identical in structure, in virtually all respects, to those described in human gastric carcinomas. The exceptions to this were the small number of microcysts observed in sheep lesions which were not surrounded by an area of cytoplasm devoid of organelles, and the very few microcysts not lined by microvilli. The means by which microcysts are formed is not known, although Kondo et al. considered they arose in the area of Golgi apparatuses, and probably from them, representing an abnormal manifestation of secretion in carcinomas. In this investigation, Golgi apparatuses were only occasionally observed immediately adjacent to microcysts, so the suggestion of Kondo et al., could not be confirmed. The similarity of the structure of microcyst walls to the brush border of both normal and some neoplastic intestinal epithelial cells suggests that they could arise by invagination of the cell surface, with subsequent closure of the cytoplasm, in a process resembling phagocytosis. Whether mucus was included in a process such as this, or whether it was secreted into the vacuoles could not be determined. Neither the present observations, nor those reported by Kondo et al., revealed signs of secretory activity in cells containing microcysts. Goblet cells of the intestinal epithelium may, however, revert to the appearance of columnar epithelium (Freeman, 1962; Cowdy, 1963), and a similar phenomenon could conceivably occur in neoplastic cells about the time of microcyst formation.

The distribution of primary lesions encountered in this study was in contrast to the distribution reported in man (page 19) and cattle (page 23). Whereas the duodenal epithelium gives rise to a large proportion of carcinomas of the small intestine in man, no

carcinomas arose in this site in sheep. In this investigation, carcinomas were found to arise from almost the whole length of the jejunum and ileum, but with a maximum frequency about the mid-portion of the small intestine and decreasing toward either end. This distribution is more extensive than previously recorded in sheep (Dodd, 1960; McDonald and Leaver, 1965; Webster, 1966).

An outstanding feature of serosal lesions was the massive proliferation of collagenous fibrous tissue to make up the great bulk of these lesions. Many neoplasms develop about, and within, themselves a stroma of supporting connective tissue. Few are described with such a massive stroma as was present in these tumours. Sampson (1931) considered cells of ovarian carcinomas free in the peritoneal cavity caused toxic or enzymic damage to the peritoneum. This resulted in the cells becoming attached to the peritoneal surface by fibrin and to be included in the consequent fibrous reaction. Coagulative properties for tissue fluids, were ascribed to cells of a wide range of human carcinomas by O'Meara (1958). It was suggested that this coagulative property of carcinomatous cells was responsible for fibrin deposition about invading squamous carcinoma cells as observed by O'Meara and Jackson (1958), and that it subsequently resulted in fibrous tissue formation and the development of other stromal components. It appears intrinsic in the suggestions of O'Meara and Jackson that neoplastic cells, at least in the squamous cell carcinomas examined by them, lost their coagulative properties as further tumour growth proceeded peripheral to them, thus limiting the formation of stroma. It might be that in the case of carcinomas of the small intestine of sheep, such coagulative properties are retained by the neoplastic cells

resulting in a continuation of fibrin deposition and subsequent collagen production. In sections examined under the electron microscope, and under the light microscope with special stains, however, collagen was present immediately adjacent to many neoplastic cells in serosal lesions and fibrin, as described by O'Meara and Jackson, was not observed. The presence, in a number of sections, of whorls of immature fibrous tissue surrounding neoplastic cells suggests that these cells were acting as a continuing stimulus to fibrous tissue formation. It seems likely that in these situations pressure necrosis of enclosed neoplastic cells might take place. This would explain the apparent decrease in numbers of neoplastic cells noted in larger serosal lesions. Factors other than coagulative properties of neoplastic cells must be involved in the stroma production, as demonstrated in this tumour by the relative lack of stroma in other than serosal lesions. Ample opportunity exists in other sites, particularly within lymph node sinuses and liver sinusoids, for the coagulation of tissue fluids, yet stroma formation in these other sites was absent or much less prolific.

Neither fibrocyte proliferation nor excessive collagen production was observed in the vicinity of mucus secreted into intercellular spaces, so there was no clear evidence that mucus was the factor promoting stromal formation.

The examination of large numbers of affected sheep has shown that these tumours spread from the primary sites in a characteristic manner. Initially there is involvement of the mesenteric lymph node draining the primary lesion and early spread occurs proximally along

the intestinal wall and mesenteric attachment of the small intestine, as well as across the mesentery toward the related lymph node. These gross observations are consistent with direct spread along lymph vessels by both metastasis and permeation. Some retrograde passage of tumour emboli and retrograde permeation from affected lymph nodes may also occur. Such retrograde embolic spread within lymphatics has been demonstrated by Zeidman (1959).

Factors restricting the distal spread of secondary lesions along the intestinal wall are obscure, though this is most probably related to differential lymph pressures. It is feasible that dilatation of lymph vessels occurs in the wall of the proximally dilated small intestine in those cases where intestinal obstruction has occurred, and that this might facilitate proximal spread. This, however, could not account for the solely proximal spread in cases in which obstruction had not occurred.

Lesions on the omentum, parietal peritoneum, diaphragm, colon, caecum, fore-stomachs, liver capsule and spleen presumably resulted from transcoelomic spread. The release of cells into the abdominal cavity would result in their anterior migration with the flow of peritoneal fluid draining through the diaphragmatic lacunae (Higgins and Graham, 1929; Yoffey and Courtice, 1956), and the seeding of many cells onto the diaphragm and anterior ventral parietal peritoneum. Lymph from the diaphragm drains to the parasternal lymph vessels and thence to the anterior mediastinal lymph nodes. The parasternal lymph vessels also have connecting vessels, running along the anterior and posterior border of each intercostal space, which connect with the dorsal mediastinal lymph nodes. Lymph drains directly from the dorsal

part of the diaphragm to the dorsal mediastinal nodes (Yoffey and Courtice, 1956). It is considered that from diaphragmatic lesions spread took place via the lymphatic routes described above.

The large volume of ascitic fluid present in most sheep in the terminal stages of this disease was considered to result from the blockage of diaphragmatic lacunae and other lymphatics draining the abdominal cavity.

The only evidence of the successful haematogenous spread of neoplastic cells was the hepatic parenchymal lesions in three animals. This scarcity of hepatic lesions contrasts with Dodd's 1960 report of several affected livers within twenty-five cases and the frequent reports of liver involvement in cases of carcinomas of the small intestine in man (Wood, 1967), cattle (Schlegel, 1925) and dogs (Rubarth, 1934; Krook, 1956). It is suggested that the establishment of hepatic metastases occurs late in the course of the disease, since in the present investigation lesions came mainly from apparently healthy slaughtered animals, whereas the other reports were based mainly on material from fatal cases. Fisher and Fisher (1965) discussed the wide range of factors affecting the rat liver making it more susceptible to the establishment of blood borne metastases. Similar factors may operate late in the course of the development of small intestinal carcinomas, perhaps as a result of chronic intestinal obstruction and interference with intestinal absorption, rendering the liver a more suitable environment for proliferation of neoplastic cells.

SUMMARY

Lesions from 450 sheep with carcinomas of the small intestine were examined grossly, and 200 of these were submitted to histological examinations. Tissues from seven lesions were examined with an electron microscope.

Apart from the degree of secondary spread, the gross appearance and histopathology of all lesions was generally similar. They arose in the jejunum - ileum, with a maximum frequency about the middle of its length. Multiple primary lesions were not uncommon. All lesions contained two predominant neoplastic cell types, one resembling columnar absorptive cells and the other goblet cells of the intestinal epithelium. It was concluded that the neoplastic cells arose from stem cells in the epithelium of the small intestine which retained the ability to differentiate to resemble either of the two cell types mentioned.

A number of cytological features regarded as characteristic of neoplastic cells by other authors were found in the cells of these carcinomas. In addition, intracytoplasmic vacuoles lined by microvilli were described. Similar structures have previously been noted only in gastric carcinomas of man. It was suggested that these structures arose by invagination of the walls of neoplastic cells.

Serosal lesions consisted largely of collagenous fibrous tissue with few neoplastic cells, particularly in larger lesions. While possible reasons for the predominance of collagenous tissue in these lesions were discussed, no firm conclusions were reached.

Secondary spread was mainly via lymph vessels, by both metastasis and permeation. Some lesions in more advanced cases were considered to have arisen by dissemination of cells within the peritoneal cavity. Haematogenous metastases were seen in the livers of only three animals, in each of which there was also extensive spread of tumour to other sites. It was considered that hepatic metastases established late in the course of development of these carcinomas.

On the basis of the remarkable similarity of lesions examined, it was considered that this series of carcinomas of the small intestine of sheep represented a single pathological entity.

C H A P T E R I I I

AN INVESTIGATION OF THE EPIDEMIOLOGY OF CARCINOMAS OF THE SMALL INTESTINE IN SHEEP

INTRODUCTION

The value of epidemiological statistics in human cancer investigations has been emphasised by World Health Organisation expert committees on at least two occasions (Wld.Hlth.Org.techn. rep.ser. No. 365 and 426). This is supported by observations on many compounds first suspected as carcinogenic in man following epidemiological observations, and later confirmed as carcinogenic by animal studies. Epidemiological investigations of human cancer have been of considerable value in assisting the formulation of hypotheses regarding the aetiology of specific tumours. Doll (1967) emphasised the value of epidemiological studies in regard to future prevention of human cancer.

The early observations concerning scrotal cancer in chimney sweeps, and the suggestion by Pott (1775) that this condition was caused by soot, were not supported by data capable of analysis by modern statistical techniques. Nevertheless, these simple observations were a revelation in Pott's time, and lead eventually to the compulsory notification of this disease in Great Britain and its legal recognition as an occupational disease.

Another classical example of the value of epidemiological observations and investigations began with the recognition by Rehn (1895)

that an unusually large number of workers in a dye factory presented to him with cancer of the bladder. Later, detailed investigations by Goldblatt (1947) and Case, Hosker, McDonald and Pearson (1954) incriminated benzidine and 2-naphthylamine as causative agents in human bladder cancer, but were indeterminate as regards the role played by 1-naphthylamine. Considerable experimental work in animals has subsequently confirmed the carcinogenicity of these industrial chemicals and certain related compounds.

The epidemiological relationship between lack of circumcision and increased susceptibility to penile cancer (Sorsby, 1931; Nath and Grewal, 1939) appears to be a relatively direct one. While the nature of the carcinogen responsible has not been determined, it is probably related to penile hygiene.

Many epidemiological investigations have followed the initial observation by Cullen (1900) that in fifty cases of carcinoma of the cervix, all patients were married and only one was childless. Direct associations between incidence of cervical carcinoma and a high frequency of intercourse, lack of penile circumcision and lack of penile and coital hygiene (Rewell, 1957; Kmet Damjanovski, Stucin, Bonta and Cakmakav, 1963; Stucin, Bonta, Kovacic, Hribar and Damjanovski, 1963; Elliott, 1964; Boyd and Doll, 1964; Aitken-Swan and Baird, 1965; Osborn and Leyshon, 1966) support an hypothesis that male smegma may contain a causal carcinogen.

Perhaps the most important and immediately applicable discovery

in the epidemiology of human cancer has been the link between tobacco smoking, especially in the form of cigarettes, and bronchogenic carcinoma. Both retrospective and prospective studies have revealed a greater preponderance of cigarette smokers amongst persons developing lung cancer. These studies also show that the relationship between cigarette smoking and lung cancer risk is quantitative, heavier smokers being more prone than light smokers (Doll and Hill, 1956; Cornfield, Haenszel, Hammond, Lilienfeld, Shimkin and Wynder 1959; Royal College of Physicians, 1962; Doll and Hill 1964). These authors and many others have held that the relationship between cigarette smoking and lung cancer must be a casual one.

The potential importance of epidemiology to the field of cancer research is further revealed by global and national mapping of the recorded incidence of particular neoplasms. The maps published by Doll (1967), together with the relevant text, emphasised the wide geographic differences in tumour incidence which exist, and drew attention to the need for greater effort directed towards finding reasons for these differences.

Many reports of apparent associations between particular neoplasms and suggested aetiological factors have been based on small numbers of patients at particular hospitals or clinics, or within localised areas. Much data collected on this scale is subjected to bias resulting from either referred patients to specialist units, or special interests of hospital staff. Data collected from surgical and/or clinical records may omit persons dying rapidly from the effects of a neoplasm, and mortality data becomes a less reliable source of material in tumours more amenable to treatment. Differences in

intensity of medical care, and in methods and enthusiasm of data collecting agencies, will obviously be reflected in differences in tumour incidence records. These factors will affect apparent geographic differences in tumour prevalence and also apparent changes of tumour prevalence with time. Regional and national cancer registries now attempt to collect data from all sources including medical, surgical and autopsy records from state and private hospitals, and individual medical practitioners. Where this is done, and where medical care is widely available to, and sought by, the population, relatively more accurate information is available.

The uneven geographic distribution of carcinoma of the stomach has stimulated a number of epidemiological investigations. Wynder(1967) discussed geographic variations in death-rates from this disease which were particularly high in Chile, Japan and Iceland and low in Mexico, Ceylon, Egypt and Caucasian United States populations. He showed that male mortality-rates exceeded female mortality-rates and that there had been a trend over recent years for carcinoma of the stomach to become less common. This trend toward decreased incidence had been most marked in females and in younger age groups. Clemmesen (1965) reviewed the factors which various authors had found associated with a higher incidence of stomach cancer. Included were particular soil types, and climatic, environmental, occupational, dietary and hereditary factors, and associations with other diseases. Unfortunately, such associations have failed to reveal any definite aetiological agents. Statistical associations between cancer incidence and other factors may be due to a causal relationship, a relationship of that factor to

some other causal factor, or merely to chance. All three possibilities must be considered before the aetiological significance of such findings can be decided. Similar relationships found in other investigations may greatly reduce the likelihood of chance occurrences continuing to be misinterpreted, but do little to discount the possibility of an indirect relationship with a causal factor. The multiplicity of factors found to be correlated with the incidence of stomach cancer by various authors, emphasises the difficulty in interpreting such findings.

Another example of the value of epidemiological studies of tumours is that of "Burkitt's Lymphoma" (Burkitt, 1958). The peculiar age distribution, lack of tribal, geological or dietary associations, and the rather precise geographic distribution of this tumour (Burkitt, 1962, 1964) lead to the hypothesis that it was caused by an arthropod-borne virus. Of particular relevance to this hypothesis was the correlation of the geographic distribution of this tumour with the distribution of known arthropod-borne diseases in Africa (Burkitt, 1964, 1967). On the basis of the geographic pathology of this tumour, Harris (1964) suggested that it was "caused by an insect-transmissible agent moving in a largely immune adult population".

In animals, much less use has been made of the epidemiological approach to the investigation of neoplasms, possibly due to the relative lack of data upon which to commence any such investigation. Few meaningful cancer recording schemes are in operation, nor do animal populations usually have the same degree of medical surveillance as humans, and precise information on the age, sex and breed structure

of many animal populations is often not available. An exception to this is the Danish cattle leukosis* registration system which Bendixen (1966) considered was working efficiently.

Data available on the prevalence of neoplasms in domestic animals come mainly from records of neoplasms diagnosed in animals slaughtered at meat processing plants, or records of neoplasms in surgical biopsy material or in animals examined post mortem at various laboratories. The population of animals slaughtered for meat is obviously a very biased one, tending to consist mainly of younger animals in good health. Older animals, when slaughtered, vary considerably in age, and life histories are rarely available. The proportion of animals in poor health slaughtered, either at abattoirs or at rendering plants, varies considerably with the value of the animals and the distance over which they must be transported. All these factors make comparisons of tumour statistics from different slaughtering establishments or countries difficult to interpret.

The prevalence of various diseases diagnosed at laboratories can be influenced greatly by the intensity of veterinary surveillance of the animal population, and by any particular interest of veterinarians utilising the laboratory facilities. Animals or tissues examined at diagnostic laboratories can be expected to represent only a small sample from the total of all diseased animals.

* A wide range of terms has been used for neoplastic diseases of cells of the lymphoid system (e.g. leukaemia, malignant lymphoma, lymphosarcoma, lymphomatosis). Following the example of Bendixen (1966) the term leukosis has been used in this thesis to cover a range of leukaemic and aleukaemic conditions which may, or may not, have a similar aetiology.

The epidemiology of enzootic bovine leukosis has been investigated in considerable detail on international, national and local levels. The occurrence of multiple incidence leukosis herds, the increased incidence of leukosis following some animal importations, the introduction of leukosis to herds with the introduction of animals from multiple incidence herds, and the apparent control of the disease by means of a slaughter policy, all support the hypothesis that this disease is caused by a transmissible agent, probably a virus (Bendixen, 1965).

Differences in the incidence of carcinoma of the tonsil between rural and urban dogs are consistent with the suggestion that these neoplasms might be caused by atmospheric pollutants (Ragland and Gorham, 1967). However, differences in the environment of these two canine groups must be manifold and the breed structure of the two populations might also differ markedly. Care should be taken in attributing such differences in incidence of neoplasms to any single environmental factor.

There have been no reports of planned epidemiological investigations of sheep neoplasms though retrospective observations on the frequency and distribution of several neoplasms have lead to suggestions regarding their aetiology. Dodd (1923) considered that excessive sunlight irritated the unprotected portions of the skin of sheep (particularly their ears) leading to chronic inflammation and eventually to squamous cell carcinoma. Lloyd (1961) supported the opinion that sunlight was the cause of this form of carcinoma and suggested an increased incidence might be associated with prior

photosensitization. The role of sunlight in the pathogenesis of these neoplasms is further supported by the observations that white-skinned people living in areas with high sunshine hours and a clear atmosphere are more prone to develop squamous cells and basal cell carcinomas of the skin (Blum, 1955).

In contrast to the above neoplasms are squamous cell carcinomas of the wool bearing area of the skin of sheep (page 6). Initial epidemiological observations that this neoplasm occurred in three related inbred Merino flocks led to the experimental confirmation that there was an indirect genetic predisposition to the development of these lesions (Carne et al., 1963; Lloyd, 1966).

Epidemiological observations of pulmonary adenomatosis in sheep first suggested a transmissible agent (page 4). Its epizootic spread in Iceland (Dungal, 1938) was particularly good epidemiological evidence of its transmissible nature.

Available data on the world incidence of small intestinal carcinomas of sheep indicate that an uneven geographic distribution may exist (Table V). On the basis of their observations, McDonald and Leaver (1965) concluded that there was an uneven incidence between flocks in the same locality. Because of the high incidence of this neoplasm in New Zealand (Dodd, 1960, 1964; Webster, 1966, 1967) an epidemiological study was undertaken in an attempt to identify factors associated with its occurrence.

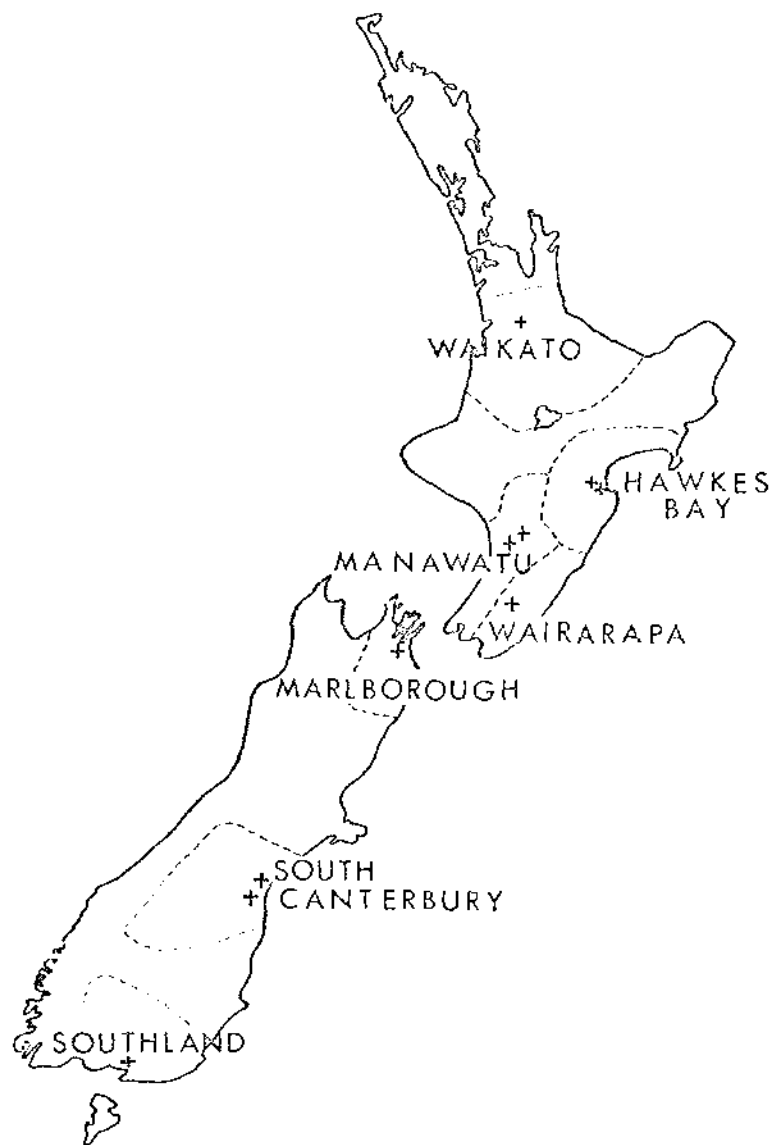


Figure 65

Map of New Zealand, showing locations of Meat Export Works (+) at which viscera of slaughtered ewes were inspected, and the approximate boundaries of the areas from which those Meat Export Works would obtain the majority of their ewes.

MATERIALS AND METHODS

Collection and Evaluation of Data

The abdominal viscera of ewes killed at Meat Export Works were examined for carcinomas of the small intestine, with particular attention being paid to the small intestine, its mesentery and mesenteric lymph nodes. The viscera were inspected on trays accompanying the slaughtering "chain" at rates of from five to seven per minute. All inspections were done by the author.

Accuracy of data collected from the gross examination of lesions was established by the histological checking of fifty consecutive lesions during 1967, and every fifth case diagnosed during 1968. Any other suspect small intestinal carcinoma for which a diagnosis was in doubt on gross examination, was checked histologically.

Geographic Distribution of Carcinomas of the Small Intestine

Ewes killed at ten Meat Export Works in seven geographical areas of New Zealand (Figure 65) between February and May of the years 1967 and 1968 were examined for carcinomas of the small intestine. Records were kept of the numbers of ewes examined and numbers of small intestinal carcinomas found at each slaughtering establishment.

Correlation of Tumour Prevalence with Average Stocking Densities in Areas

Areas available for sheep and beef cattle grazing in counties considered representative of areas supplying the majority of sheep to

Meat Export Works at which ewes were inspected were calculated. This was done by obtaining an estimate of the total grazing land (acres) in each county and subtracting from that figure an estimate of the area in each county occupied by dairy farms. Information in "New Zealand Farm Production for the season 1967 - 1968"* was used in applying the following formulae : -

$$\begin{array}{l}
 \left(\begin{array}{l} \text{Area for sheep} \\ \text{and beef} \\ \text{cattle grazing} \\ \text{in individual} \\ \text{North Island} \\ \text{areas.} \end{array} \right) \text{ equals } \left(\begin{array}{l} \text{Area in grass,} \\ \text{clover and} \\ \text{lucerne in} \\ \text{area.} \end{array} \right) \text{ minus } 1\frac{1}{2} \left(\begin{array}{l} \text{Dairy cows} \\ \text{in milk} \\ \text{in area.} \end{array} \right)
 \end{array}$$

and

$$\begin{array}{l}
 \left(\begin{array}{l} \text{Area for sheep} \\ \text{and beef} \\ \text{cattle grazing} \\ \text{in individual} \\ \text{South Island} \\ \text{areas.} \end{array} \right) \text{ equals } \left(\begin{array}{l} \text{Area in grass,} \\ \text{clover and} \\ \text{lucerne in} \\ \text{area.} \end{array} \right) \text{ plus } \frac{2}{3} \left(\begin{array}{l} \text{Balance} \\ \text{of land} \\ \text{in area} \end{array} \right) \text{ minus } \frac{1}{2} \left(\begin{array}{l} \text{Dairy} \\ \text{cows} \\ \text{in milk} \\ \text{in area} \end{array} \right)
 \end{array}$$

These formulae were based on assumptions that the area in grass, clover and lucerne would approximately equal the total area available for livestock grazing in the North Island, and that the area (acres) occupied by dairy farms would approximate $1\frac{1}{2}$ times the number of dairy cows in milk. The formula used to calculate the areas over which sheep and beef cattle were grazed in the South Island was different in that $\frac{2}{3}$ of the "balance of land" in each area was included, this being an estimate of the area of unimproved tussock grasslands. Also, because

* Government Printer, Wellington, New Zealand.

T A B L E XII

Counties Used in Calculations of Average Stock Grazing
Densities in Areas From Which Inspected Ewes Originated

Area	Breed type of sheep	Counties
Hawkes Bay	British breed	Wairoa, Hawkes Bay, Waipawa, Waipukurau, Patangata, Dannevirke
Manawatu	British breed	Kiwitea, Pohangina, Oroua, Manawatu, Kairanga, Woodville, Pahiatua
Waikato	British breed	Raglan, Waikato, Hauraki Plains, Piako, Ohinemuri, Tauranga, Matamata, Rotorua, Taumaranui, Waitomo, Otorohanga
South Canterbury (a)	British breed	Levels, Waimate
Southland	British breed	Clutha
Wairarapa	British breed	Eketahuna, Masterton Wairarapa South, Featherston
Marlborough	Fine wool	Amuri
South Canterbury (b)	Fine wool	MacKenzie

dairy farming is less intensive in the South Island than in the North, an area of 2 times the number of dairy cows in milk was subtracted as being occupied by dairy farms.

Using these figures, and data from the same publication on total sheep and beef cattle numbers in the respective counties, average numbers of sheep and beef cattle per acre of available grazing were calculated. Counties used for these calculations are shown in Table XII.

Effect of Environment on Prevalence of Small Intestinal Carcinomas

Between mid January and early June 1969, the viscera of 32,733 ewes were examined for carcinomas of the small intestine as previously described. During this period, details of the number of ewes in each group (line) inspected, and their vendors, were obtained and tumours were identified with the lines from which they came. Most ewes inspected were slaughtered in the Manawatu region, although a small number (2356) were inspected in the Wairarapa (See Figure 65).

All sheep vendors who could be traced were forwarded a questionnaire (Appendix 1) with a covering letter explaining the purpose of the investigation. At a later date an appointment was arranged and the questionnaire was completed by the author with the cooperation of the sheep vendor. Information was obtained only from vendors who had owned the inspected sheep for longer than six months. In this way, data were obtained on the histories of 22,201 of the inspected ewes, forwarded by 191 vendors.

Physiographic and Botanical Data
Concerning Farms Investigated

(i) Soil Types

Soil types constituting at least 20% of each farm from which ewes were inspected were assessed from soil maps of New Zealand (Scale 4 miles to an inch) used in conjunction with Soil Bureau Bulletin (N.S.) 5 - General Survey of the soils of the North Island, New Zealand (1954)*. Classification of soil types for the purpose of analyses was on the basis of numbers only, without the alphabetical suffix used in the bulletin and on the maps. This resulted in some grouping of soil types as shown in Appendix II.

(ii) Climate

Information on rainfall was obtained from a map (scale 4.9 miles to an inch) supplied by the New Zealand Weather Bureau showing $2\frac{1}{2}$ inch isohyets and based on data collected between 1921 and 1950.

Average summer maximum and average winter minimum temperatures at recording stations were calculated from the New Zealand Department of Civil Aviation publication, N.Z. Meteorological Service Miscellaneous Publication 122 (1966)** by averaging the mean daily maximum figures for December, January and February, and the mean daily minimum figures for June, July and August. Temperature data pertaining to the recording station nearest each farm was adjusted where necessary by subtracting 0.35°F for each 100 feet by which the mean farm altitude exceeded the altitude of the recording station (Robertson, 1969).

* Department of Scientific and Industrial Research, Wellington, New Zealand.

** New Zealand Meteorological Service, Wellington, New Zealand.

(iii) Altitude and Latitude

The approximate mean altitude of each farm was estimated from maps (New Zealand Mapping Service (N.Z.M.S.) 1, scale 1:63,360,* and N.Z.M.S. 18, scale 1:250,000*).

The latitude of farms was similarly obtained from maps (N.Z.M.S. 9, scale 1:500,000)*.

(iv) Plant Species

Pasture species and weed species occurring on farms were recorded following discussions with individual farmers. To assist this, a check list of weed species was used (Appendix III), but other plant species not on this list were also recorded (Appendix IV).

Managerial factors

(i) Stock Management

When sheep slaughtered were not an homogeneous group, answers to questions covering breed, age and reasons for slaughter were based on the answers applicable to the majority of animals in the line.

"Mob stocking" was considered to be the practice where sheep were concentrated into paddocks and a system of rotational grazing about the farm was instituted, or where sheep were gathered into relatively small areas for the feeding of crops or other supplementary food. In contrast, "set stocking" referred to the practice whereby the sheep were grazed over all, on most, of the available pasture for most of the time.

* Department of Lands and Survey, Wellington, New Zealand.

(ii) Animal Remedies and Agricultural Chemicals Used

Questions on animal insecticides, anthelmintics, Clostridial vaccines, stock licks and selenium administration were answered on the basis of exposure of the inspected sheep to these agents during the time the vendor had owned them. Types of Clostridial vaccines or types of stock licks were not differentiated between.

Applications of dichlorodiphenyl trichloroethane (DDT) to pasture over the previous seven years were recorded, but applications of other pasture insecticides and of herbicides were recorded only if they had occurred during the time the slaughtered sheep were on the property, or during the 12 months preceeding their purchase.

(iii) Supplementary food

Details of the supplementary feed made available to sheep during their lives on the vendors property were recorded from only 11,378 sheep as this question was inserted after the investigation had begun.

Flock Disease History

Classifications of farms with regard to sporidesmin poisoning (facial eczema) and Salmonellosis occurrence were subjective. Diagnosis was frequently based only on farmer opinion.

Analysis of Data

Tables XIII, XIV, XV, XVI, XXV and XXVII were analysed by the chi squared method for 2 x 2 and 2 x j contingency tables as appropriate (Mather, 1960). Analyses in Table XVII were carried out using the method for 2 x 2 contingency tables, and data in Tables XXI and XXIII

were variously treated as 2 x 2 and 2 x j contingency tables.

Data in Table XX were analysed for heterogeneity using the formula

$$X_{f-1}^2 = W\phi^2 - \frac{(W\phi)^2}{W} \quad (\text{Hayman, 1968})$$

where f = number of sheep vendors

$$W = \frac{n}{820.7} \quad (n = \text{number of sheep from vendor})$$

and ϕ = inverse sine transformation of the percentage (p) of sheep in that line in which tumours were diagnosed. (Derived from Table X in Fisher and Yates (1957) or where $p < 4$; $\phi = 57.3\sqrt{.0p}$)

Cross tabulations of data in Table XXIII were carried out on a computer as also were the chi squared and probability calculations in the table.

Table XVIII was analysed by both single and multiple regression techniques (Mather, 1960) and the significance of the inter-relationships was calculated using analysis of variance techniques (Table XIX).

RESULTS

Collection and Evaluation of Data

The accuracy of diagnoses made from gross examinations of intestines was confirmed by positive histological diagnoses in all fifty consecutive check cases in 1967, and in every fifth lesion diagnosed grossly as carcinoma of the small intestine during 1968 (49 check cases). In no case was a histological diagnosis of carcinoma made from any lesion that had not been diagnosed with

T A B L E X I I I

Numbers of Ewes Examined and Small Intestinal Carcinomas Found
in Seven Geographic Areas of New Zealand During 1967 and 1968

Area	1967		1968				Total
	Ewes examined	Carcinomas found	Ewes examined	Carcinomas found	Ewes examined	Carcinomas found	Percentage of ewes with Carcinomas
Hawkes Bay	-	-	4,000	63	4,000	63	1.58
Manawatu	4,000	48	4,000	71	8,000	119	1.49
Waikato	2,600	39	-	-	2,600	39	1.50
South Canterbury	2,600	13	4,200	31	6,800	44	0.65
Southland	-	-	4,000	41	4,000	41	1.03
Wairarapa	1,900	19	4,000	34	5,900	53	0.90
Marlborough	-	-	4,000	8	4,000	8	0.20

$$\chi^2 = 69.00; \text{ d.f. } = 6; \text{ p } < 0.001$$

T A B L E XIV

Carcinomas of the Small Intestine Found in British Breed
and "Fine Wool" Sheep Inspected at South Canterbury During 1968

Breed type	Ewes inspected	Carcinomas found	Percentage with carcinomas
British breeds	2700	26	0.96
"Fine wool" breeds	1500	5	0.33

$$\chi^2 = 5.22; \text{ d.f. } = 1; p < 0.05$$

T A B L E XV

Carcinomas of the Small Intestine Found in Sheep Identified as
British Breed and "Fine Wool" Sheep Inspected During 1967 and 1968

Breed type	Ewes inspected	Carcinomas found	Percentage with carcinomas
British breeds	27,200	341	1.25
"Fine wool" breeds	5,500	13	0.24

$$\chi^2 = 44.55; \text{ d.f. } = 1; p < 0.001$$

T A B L E XVI

Carcinomas of the Small Intestine Found in British
Breed Sheep Inspected in Different Districts of
New Zealand During 1967 and 1968

District	Ewes inspected	Carcinomas found	Percentage with carcinomas
Hawkes Bay	4,000	63	1.58
Manawatu	8,000	119	1.49
Waikato	2,600	39	1.50
South Canterbury	2,700	26	0.96
Southland	4,000	41	1.03
Wairarapa	5,900	53	0.90

$$\chi^2 = 16.96; \text{ d.f. } = 5; p < 0.01$$

T A B L E XVII

χ^2 figures and Probabilities (p) for Differences Between
Tumour Incidences in British Breed Sheep From Different Areas

	Hawkes Bay	Manawatu	Waikato	South Canterbury	Southland
Manawatu	$\chi^2 = 0.13$ N.S.				
Waikato	$\chi^2 = 0.6$ N.S.	$\chi^2 = 0.02$ N.S.			
South Canterbury	$\chi^2 = 4.58$ $p < 0.05$	$\chi^2 = 4.10$ $p < 0.05$	$\chi^2 = 3.1$ N.S.		
Southland	$\chi^2 = 4.65$ $p < 0.01$	$\chi^2 = 4.32$ $p < 0.01$	$\chi^2 = 2.95$ $p < 0.02$	$\chi^2 = 0.08$ N.S.	
Wairarapa	$\chi^2 = 0.28$ $p < 0.01$	$\chi^2 = 9.52$ $p < 0.01$	$\chi^2 = 5.96$ $p < 0.02$	$\chi^2 = 0.08$ N.S.	$\chi^2 = 0.59$ N.S.

reasonable certainty as an intestinal carcinoma on prior gross examination. On the basis of these checks, it was considered that diagnosis of carcinoma of the small intestine by gross examination only was an accurate method of accumulating data on the prevalence of these neoplasms.

Geographic Distribution of Carcinomas of the Small Intestine

The prevalence of small intestinal carcinomas diagnosed in slaughtered ewes inspected in each of the seven geographic areas visited (Figure 65) is shown in Table XIII. Calculation of chi squared on the "total" data for each district showed the distribution of tumours between areas was not normal ($p < .001$). From Table XIII it is apparent that the prevalence of small intestinal carcinomas in sheep inspected in Marlborough was considerably lower than elsewhere, and that the prevalence in South Canterbury was also comparatively low. It was noted that at Marlborough all ewes inspected were of Merino or Corriedale ("fine wool") breeds. South Canterbury was the only other area where sheep of these breeds were inspected. Separation of data on the basis of breed types was obtained at South Canterbury during 1968 (Table XIV). A comparison of tumour prevalence in British breeds and "fine wool" breeds using data collected in 1967 and 1968 is shown in Table XV. Analyses of data in Table XIV and XV indicate a significantly lower tumour prevalence in "fine wool" sheep than in those of British breeds ($p < .05$ and $p < .001$).

Analysis of data in Table XVI, which shows the prevalence of carcinomas of the small intestine in British breed sheep examined in

T A B L E XVIII

Sheep and Beef Cattle Stocking Rates and Tumour *
Prevalence in Areas from which Ewes were Inspected

	Average number of sheep per acre (x)	Average number of cattle per acre (w)	Tumour prevalence (%) (y)
Hawkes Bay	3.4674	.3305	1.58
Manawatu	3.9756	.3125	1.49
Waikato	4.0663	.4489	1.50
South Canterbury (a) (British Breeds)	2.3806	.0543	0.96
Southland	3.4150	.1084	1.03
Wairarapa	3.0311	.2871	0.90
Marlborough	1.0769	.0506	0.20
South Canterbury (b) ("Fine wool" Breeds)	0.7739	.0249	0.50

(1) regression tumour prevalence (y) on average sheep per acre (x).

$$y = .029 + .3591 x \quad (n = 8; p < .01)$$

(2) regression tumour prevalence (y) on average cattle per acre (w).

$$y = .524 + 2.4802 w \quad (n = 8; p < .05)$$

(3) multiple regression tumour prevalence (y) on both sheep (x)
and cattle (w) stocking rates.

$$y = .085 + .2817 x + .7849 w \quad (n = 8; p < .01)$$

* In a number of tables in this chapter, the word tumour is used, for brevity, when referring to carcinomas of the small intestine.

T A B L E XIXAnalysis of Significance of the Association of Cattle
and Sheep Stocking Rates with Tumour Prevalence

(A)

	Sum of squares	Degrees of freedom	Mean Square
Regression on sheep	1.433	1	1.433
Regression on cattle after allowing for sheep effect	.047	1	.047
Error	.243	5	.049
Total	1.723	7	

$$t^2 = \frac{.047}{.049}; \quad t = .979; \quad \text{N.S.}$$

∴ There is no cattle effect on tumour prevalence that is different from the sheep effect.

(B)

	Sum of squares	Degrees of freedom	Mean Square
Regression on cattle	1.118	1	1.118
Regression on sheep after allowing for cattle effect	.362	1	.362
Error	.243	5	.049
Total	1.723	7	

$$t^2 = \frac{.362}{.049}; \quad t = 2.718; \quad p < .05$$

∴ There is an association between sheep stocking rate and tumour prevalence that is independent of cattle stocking rate.

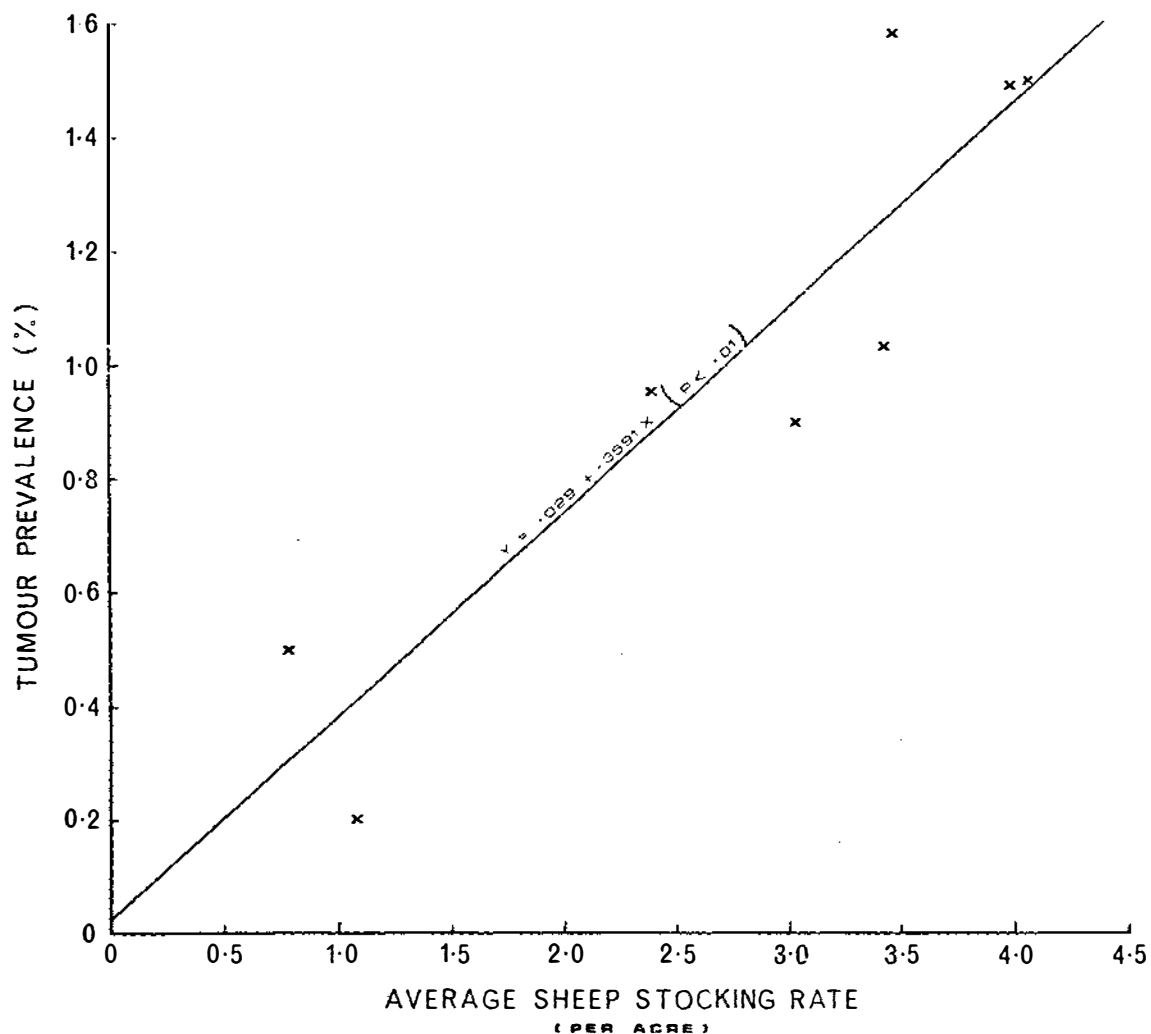


Figure 66

The relationship between prevalence of carcinomas of the small intestine in sheep slaughtered in different areas and the average sheep stocking rate in representative counties.

each district, indicates a heterogeneous distribution of tumours between sheep in different areas ($p < .01$). Table XVII shows the chi squared figures and probabilities of differences between individual areas.

Correlation of Prevalence of Carcinomas of the Small
Intestine with Stock Density

Average sheep and beef cattle stocking rates in counties considered representative of areas from which ewes were inspected (Figure 65 and Table XII) are shown in Table XVIII, together with the relevant prevalences of small intestinal carcinomas (%). Calculation of regression coefficients for tumour prevalence on average stocking rates of either sheep or cattle indicated significant positive relationships (Table XVIII). Multiple regression techniques showed there was a significant relationship between both sheep and cattle stocking rates per acre and the incidence of small intestinal carcinomas. However, calculations of the significance of each of these effects, after allowing for the other, showed that while sheep per acre had a significant relationship with tumour prevalence after allowing for any effect of cattle density, the reverse was not true (Table XIX). Thus, increased density of sheep within an area appears to be associated with increased tumour prevalence and this association is independent of cattle density. This relationship is shown in Figure 66. The relationship between cattle density and tumour prevalence was apparently due to the increased numbers of cattle in areas with higher sheep densities.

T A B L E XXNumbers of Ewes Inspected FromIndividual Sheep Vendors

Vendors code Number	Number of ewes inspected	Number of small intestinal carcinomas diagnosed	Vendors code Number	Number of ewes inspected	Number of small intestinal carcinomas diagnosed
1	195	2	30	384	3
2	354	8	31	92	1
3	38	0	32	419	8
4	8	0	33	104	0
5	79	1	35	24	0
6	252	4	37	142	4
7	127	3	38	138	2
8	156	4	39	409	9
9	86	2	40	164	1
10	22	0	44	165	1
11	101	1	46	99	3
12	75	0	47	139	1
13	41	0	49	100	2
14	32	0	50	83	1
15	13	1	51	29	0
16	67	2	52	143	3
17	223	5	53	35	0
18	10	0	55	131	1
19	30	0	56	52	1
20	91	0	57	35	0
21	38	0	59	163	2
22	275	5	60	182	3
24	43	0	61	21	0
26	19	0	62	109	2
27	290	6	63	67	0
28	5	0	64	144	2
29	53	0	66	17	0

Table XX Contd.

Numbers of Ewes Inspected From
Individual Sheep Vendors

Vendors code Number	Number of ewes inspected	Number of small intestinal carcinomas diagnosed	Vendors code Number	Number of ewes inspected	Number of small intestinal carcinomas diagnosed
67	51	0	105	165	2
69	64	0	106	28	0
70	83	0	107	89	3
71	114	1	108	16	0
72	11	0	109	24	0
73	39	0	110	103	1
75	175	1	112	13	0
76	88	0	113	424	4
77	119	1	114	114	0
78	219	6	115	189	7
79	5	0	116	69	0
80	109	2	118	125	1
82	191	5	119	79	1
83	52	1	120	140	5
84	206	3	121	37	0
85	50	0	122	85	0
87	42	1	123	15	0
88	120	4	124	137	4
89	67	2	125	50	0
91	215	1	126	26	0
92	168	3	127	166	1
93	41	0	128	46	0
95	45	0	129	12	0
96	81	2	130	153	1
97	305	3	131	142	1
100	79	4	132	185	1
102	18	1	133	140	4
103	713	10	134	117	1

Table XX Contd.

Numbers of Ewes Inspected From
Individual Sheep Vendors

<u>Vendors code Number</u>	<u>Number of ewes inspected</u>	<u>Number of small intestinal carcinomas diagnosed</u>	<u>Vendors code Number</u>	<u>Number of ewes inspected</u>	<u>Number of small intestinal carcinomas diagnosed</u>
135	209	1	169	486	9
136	88	1	170	207	2
139	116	0	172	315	7
140	6	0	174	22	1
141	27	3	175	116	1
142	45	2	176	21	1
143	72	0	177	12	0
144	246	3	178	109	0
145	70	1	179	95	0
146	40	0	180	50	0
147	152	3	181	88	3
149	126	0	182	88	1
150	140	0	183	428	11
151	18	0	185	166	4
152	243	5	186	185	0
154	138	3	188	26	1
155	8	0	189	209	6
156	235	2	190	252	8
157	161	1	191	56	0
158	83	3	192	86	1
159	102	3	193	101	1
160	38	0	194	424	13
162	41	1	195	33	1
163	134	2	196	12	0
164	89	1	200	227	6
165	293	3	201	134	0
166	131	0	202	319	6
168	33	0	203	132	4

Table XX Contd

Numbers of Ewes Inspected From
Individual Sheep Vendors

Vendors code Number	Number of ewes inspected	Number of small intestinal carcinomas diagnosed	Vendors code Number	Number of ewes inspected	Number of small intestinal carcinomas diagnosed
204	129	2	219	59	0
206	160	2	221	42	2
207	182	4	222	160	0
208	130	2	223	18	0
209	41	0	224	16	2
210	36	0	225	12	0
211	239	4	226	55	0
212	123	3	227	19	0
215	69	3	228	181	5
216	33	0	230	228	8
217	42	2	232	71	1
218	13	1	233	20	1

χ^2 Total = 358.32; d.f. = 190; $p < .001$

χ^2 (whole life history known) = 122.93; d.f. = 71; $p < .001$

χ^2 (history known for 4 years plus) = 53.33; d.f.= 33; $p < .05$

χ^2 (history known for less than 4 years) = 164.152; d.f.= 84; $p < .001$

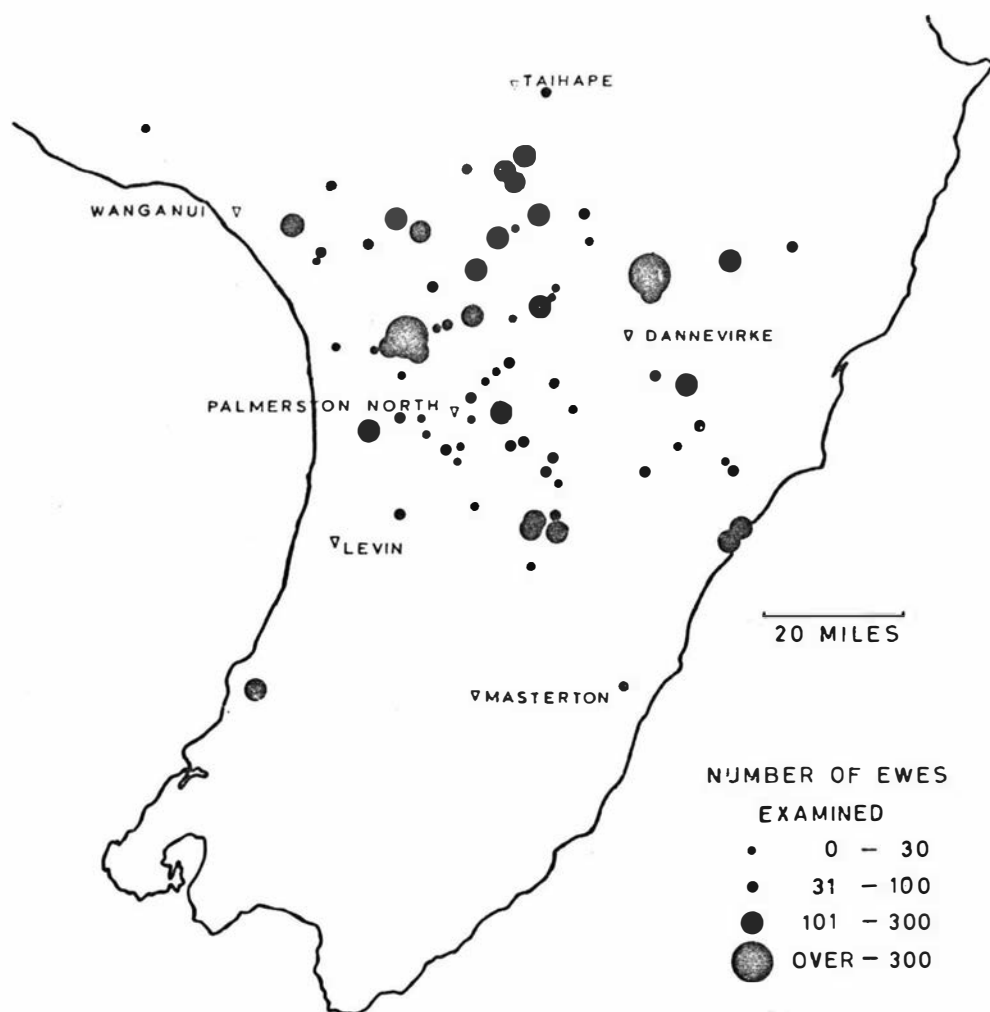


Figure 67

The locations of farms from which ewes were inspected
having been bred on those farms.

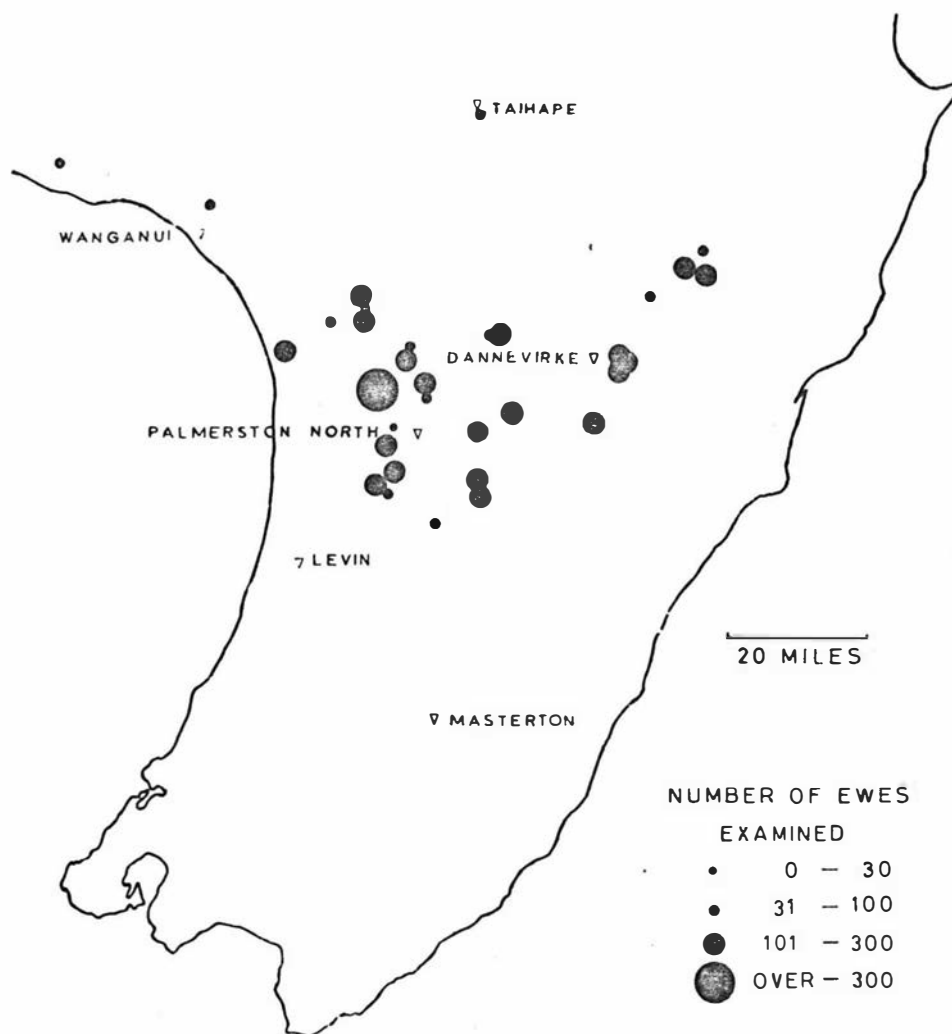


Figure 68

The locations of farms from which ewes were inspected having been purchased by the vendor at least four years previously.

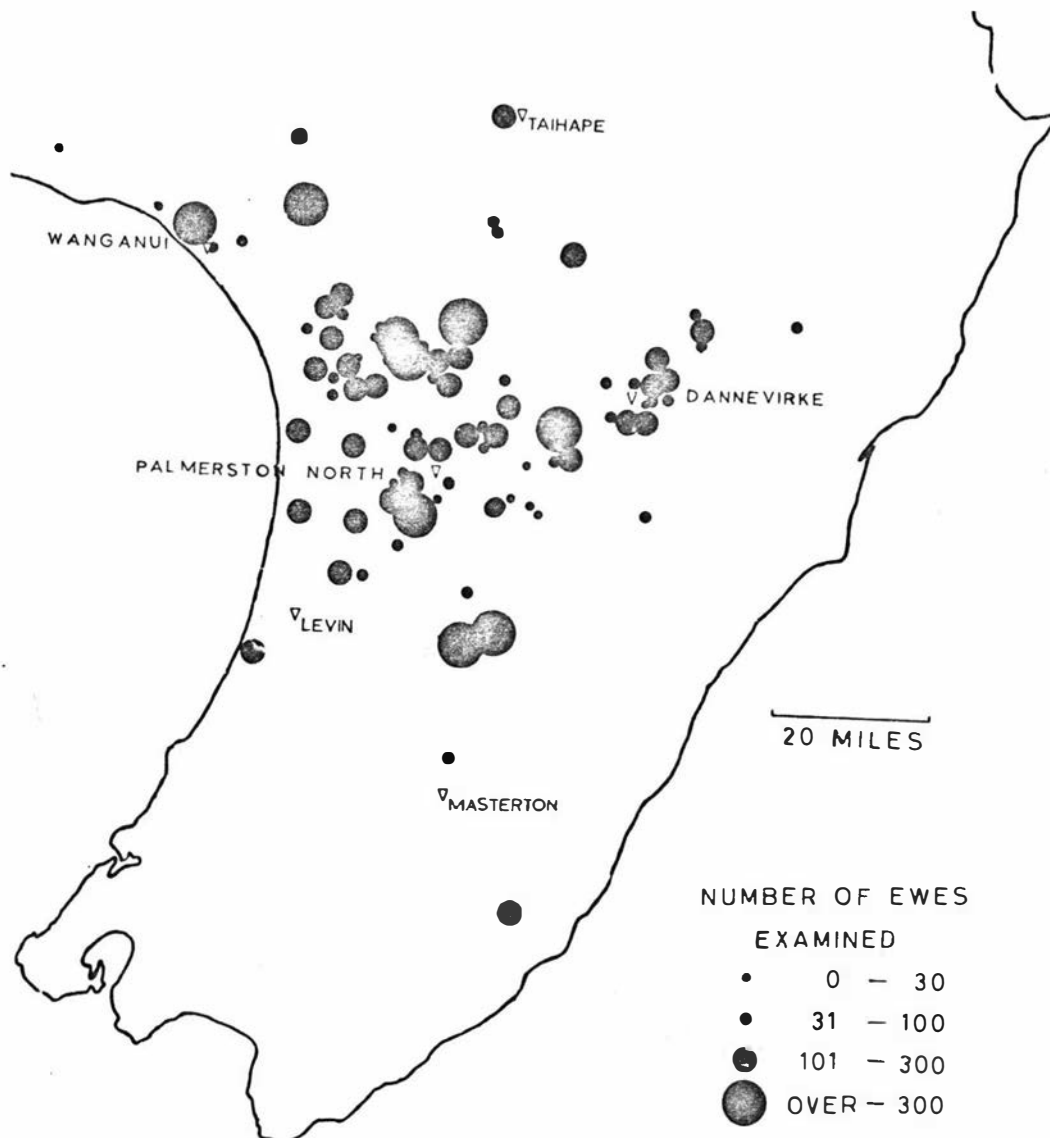


Figure 69

The locations of farms from which ewes were inspected
having been purchased by the vendor less than four years previously.

The Effect of Environment on Prevalence of Small
Intestinal Carcinomas

During 1969, a total of 32,733 ewes was examined from 294 lines, and carcinomas of the small intestine were diagnosed in 483 (1.476%) animals. Of these, historical information was obtained on 22,201 animals with 346 (1.514%) neoplasms of the small intestine, submitted by 191 vendors. Table XX lists the numbers of ewes inspected from individual vendors and the numbers of intestinal carcinomas diagnosed in those ewes. Chi squared analysis showed there was a heterogeneous distribution of tumours between the sheep of different vendors ($p < .001$).

Figures 67, 68 and 69 map the locations of farms from which ewes were inspected. Figure 67 shows the locations of farms from which ewes bred on those farms were inspected (i.e. their whole life history was obtainable from the vendor). Figure 68 maps the locations of farms from which ewes were inspected whose histories were obtainable for a period of four years or more (i.e. the ewes had not been bred by the vendor, but had been farmed by him for at least four years). Ewes from farms mapped in Figure 69 had been owned by the vendor for less than four years, but more than six months, and consequently shorter portions of their life histories were known.

Calculations of chi squared for the prevalence of small intestinal carcinomas in lines of sheep falling into each of these categories showed there was significant variation of tumour prevalence between farms within each of these classifications (Table XX).

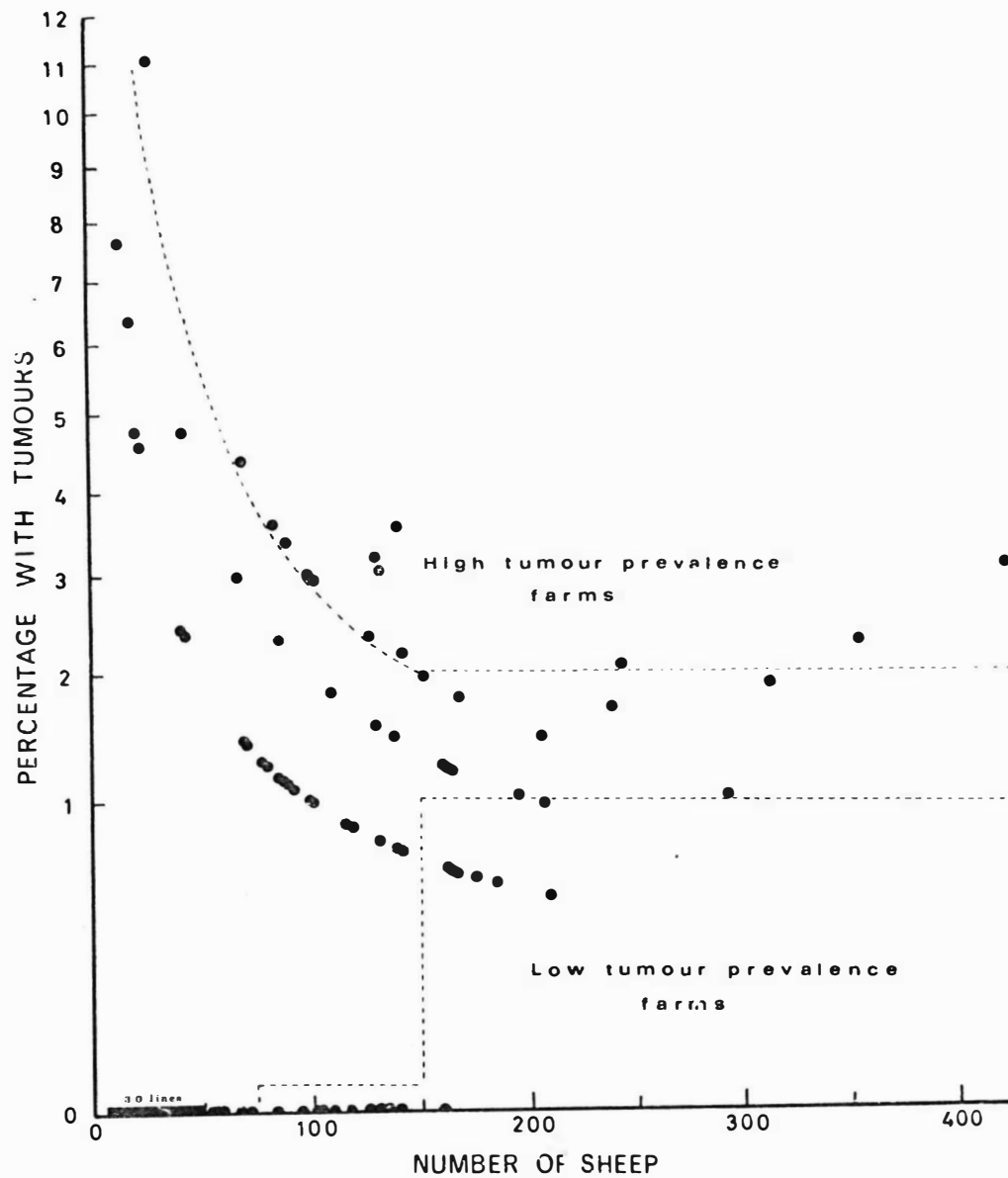


Figure 70

The numbers of ewes inspected, and the percentage of these ewes with carcinomas of the small intestine, from vendors who had owned the ewes for whole of life or at least four years.

T A B L E XXI

Tabulations of Farms with High, and Low, Prevalence of Carcinomas
of the Small Intestine According to Known Exposure to Various
Physiographic, Botanical and Managerial Factors

<u>SOIL TYPES</u>	Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Farms without <u>Soil Type 1</u>	12	17
Farms with Soil Type 1	2	1
Farms without <u>Soil Type 2</u>	13	17
Farms with Soil Type 2	1	1
Farms without <u>Soil Type 8</u>	14	17
Farms with Soil Type 8	0	1
Farms without <u>Soil Type 9</u>	14	17
Farms with Soil Type 9	0	1
Farms without <u>Soil Type 12</u>	13	16
Farms with Soil Type 12	1	2
Farms without <u>Soil Type 13</u>	11	16
Farms with Soil Type 13	3	2
Farms without <u>Soil Type 23</u>	13	18
Farms with Soil Type 23	1	0
Farms without <u>Soil Type 29</u>	13	16
Farms with Soil Type 29	1	2
Farms without <u>Soil Type 31</u>	14	17
Farms with Soil Type 31	0	1
Farms without <u>Soil Type 77</u>	10	17
Farms with Soil Type 77	4	1
Farms without <u>Soil Type 78</u>	13	18
Farms with Soil Type 78	1	0

Table XXI Contd

<u>Soil Type</u>	Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Farms without <u>Soil Type 106</u>	14	17
Farms with Soil Type 106	0	1
Farms without <u>Soil Type 114</u>	12	16
Farms with Soil Type 114	2	2
Farms without <u>Soil Type 115</u>	14	15
Farms with Soil Type 115	0	3
Farms without <u>Soil Type 117</u>	12	18
Farms with Soil Type 117	2	0
Farms without <u>Soil Type 120</u>	14	16
Farms with Soil Type 120	0	2
Farms without <u>Soil Type 122</u>	14	16
Farms with Soil Type 122	0	2
Farms without <u>Soil Type 124</u>	13	18
Farms with Soil Type 124	1	0
Farms without <u>Soil Type 125</u>	14	17
Farms with Soil Type 125	0	1
Farms without <u>Soil Type 76</u>	12	13
Farms with Soil Type 76	2	5
Farms without <u>Soil Type 27</u>	14	17
Farms with Soil Type 27	0	1

CLIMATE

Annual <u>average temperature</u> ($^{\circ}\text{F}$)	< 46	1	0
on property	46 - 49.9	0	0
	50 - 53.9	10	11
	> 54	3	7
Average <u>winter minimum</u>	< 32	1	0
temperatures ($^{\circ}\text{F}$) on property	32 - 35.9	1	1
	36 - 39.9	12	14
	> 40	0	3

Table XXI Contd

<u>Climate</u>		Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Average <u>summer maximum</u>	< 65	1	0
<u>temperature</u> (°F) on	65 - 68.9	6	3
property.	69 - 71.9	7	14
	> 72	0	1
Average <u>annual rainfall</u>	> 40	5	4
(inches) on property.	41 - 60	8	10
	61 - 80	1	4

ALTITUDE AND LATITUDE

Mean <u>altitude</u> (feet above	< 200	1	2
sea level) of farm.	200 - 499	6	4
	500 - 999	1	8
	1000 - 1999	6	4
<u>Latitude</u> (degrees	< 39° 30'	1	0
south) of property.	39° 30' - 40°	2	2
	40° - 40° 30'	9	10
	40° 30' - 41°	2	6

PASTURE SPECIES

Farms without <u>cocksfoot</u>	5	7
Farms with cocksfoot	9	11
Farms without <u>danthonia</u>	11	11
Farms with danthonia	3	7
Farms without <u>browntop</u>	4	7
Farms with browntop	10	11
Farms without <u>dogstail</u>	1	9
Farms with dogstail	13	9

$$\chi^2 = 6.73; \quad p < .05$$

Table XXI Contd

<u>Pasture Species</u>		Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Farms without <u>red clover</u>		11	11
Farms with red clover		3	7
Farms without <u>subterranean clover</u>		5	6
Farms with subterranean clover		9	12
Farms without <u>Lotus major</u>		11	12
Farms with Lotus major		3	6
 <u>WEED SPECIES</u>			
Prevalence of <u>bracken</u>	Not present	8	14
<u>fern</u> on property.	Uncommon	3	4
	Common	3	0
Prevalence of <u>other</u>	Not present	9	15
<u>ferns</u> on property.	Uncommon	5	3
Prevalence of <u>ragwort</u>	Not present	7	6
on property.	Uncommon	5	10
	Common	2	2
Farms without <u>Barberry</u>		13	17
Farms with Barberry		1	1
Farms without <u>Barley grass</u>		9	8
Farms with Barley grass		5	10
Farms without <u>Blackberry</u>		12	17
Farms with Blackberry		2	1
Farms without <u>Broom</u>		14	17
Farms with Broom		0	1
Farms without <u>Buttercup</u>		13	15
Farms with Buttercup		1	3
Farms without <u>Cape Weed</u>		14	17
Farms with Cape Weed		0	1

Table XXI Contd

<u>Weed Species</u>	Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Farms without <u>Chickweed</u>	14	17
Farms with Chickweed	0	1
Farms without <u>Couch</u>	12	17
Farms with Couch	2	1
Farms without <u>Dandelion</u>	7	9
Farms with Dandelion	7	9
Farms without <u>Daisy</u>	13	12
Farms with Daisy	1	6
Farms without <u>Dock</u>	10	11
Farms with Dock	4	7
Farms without <u>Fathen</u>	11	11
Farms with Fathen	3	7
Farms without <u>Fennel</u>	13	18
Farms with Fennel	1	0
Farms without <u>Foxglove</u>	11	13
Farms with Foxglove	3	5
Farms without <u>Gorse</u>	13	16
Farms with Gorse	1	2
Farms without <u>Hawkweed</u>	13	17
Farms with Hawkweed	1	1
Farms without <u>Hemlock</u>	11	15
Farms with Hemlock	3	3
Farms without <u>Lambs tongue</u>	14	17
Farms with Lambs tongue	0	1
Farms without <u>Linseed</u>	13	18
Farms with Linseed	1	0
Farms without <u>Lupin</u>	13	17
Farms with Lupin	1	1

Table XXI Contd

<u>Weed Species</u>	Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Farms without <u>Native bush</u>	8	14
Farms with Native bush	6	4
Farms without <u>Black nightshade</u>	12	15
Farms with Black nightshade	2	3
Farms without <u>Penny royal</u>	6	9
Farms with Penny royal	8	9
Farms without <u>Plantain</u>	14	16
Farms with Plantain	0	2
Farms without <u>Rats tail</u>	14	14
Farms with Rats tail	0	4
Farms without <u>Rushes</u>	5	9
Farms with Rushes	9	9
Farms without <u>Spurry</u>	14	16
Farms with Spurry	0	2
Farms without <u>Stinging nettle</u>	10	10
Farms with Stinging nettle	4	8
Farms without <u>Storks bill</u>	14	17
Farms with Storks bill	0	1
Farms without <u>Tall fescue</u>	14	17
Farms with Tall fescue	0	1
Farms without <u>Californian thistle</u>	3	4
Farms with Californian thistle	11	14
Farms without <u>Scotch thistle</u>	3	4
Farms with Scotch thistle	11	14
Farms without <u>Swamp thistle</u>	11	16
Farms with Swamp thistle	3	2
Farms without <u>Variegated thistle</u>	13	15
Farms with Variegated thistle	1	3
Farms without <u>Wing thistle</u>	8	13
Farms with Wing thistle	6	5

Table XXI Contd

<u>Weed Species</u>		Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Farms without <u>Trefoil</u>		13	14
Farms with Trefoil		1	4
Farms without <u>Watercress</u>		13	13
Farms with Watercress		1	5
Farms without <u>Wild carrot</u>		13	18
Farms with Wild carrot		1	0
Farms without <u>Wild turnip</u>		14	16
Farms with Wild turnip		0	2
Farms without <u>Willow weed</u>		11	14
Farms with Willow weed		3	4
Farms without <u>Wire weed</u>		13	18
Farms with Wire weed		1	0
Farms without <u>Yarrow</u>		12	15
Farms with Yarrow		2	3
Farms without <u>Yorkshire fog</u>		12	11
Farms with Yorkshire fog		2	7
Farms without <u>Land cress</u>		13	18
Farms with Land cress		1	0
Farms without <u>Mallow</u>		14	17
Farms with Mallow		0	1
Farms without <u>Ink weed</u>		14	17
Farms with Ink weed		0	1
STOCK MANAGEMENT			
<u>Sheep breeds</u>	- Romney	12	16
	Romney cross	2	2
<u>Age of sheep slaughtered</u>	< 5	1	1
(years)	5	3	4
	6	8	11
	7	1	2
	> 7	1	0

Table XXI Contd

<u>Stock Management</u>		Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
<u>Reason sheep slaughtered</u>			
Age		1	2
Tooth conditions		12	12
Poor condition		0	1
Infertility		0	2
Miscellaneous		0	1
<u>Size of farm (acres)</u>			
	100 - 499	9	9
	500 - 1499	4	5
	1500	1	4
<u>Number of ewes</u>	500 - 1499	9	11
<u>farmed</u>	1500 - 4999	5	7
<u>Average sheep stocking</u>	< 2	0	2
<u>rate (per acre)</u>	2 - 3	2	2
	3 - 5	8	9
	5 - 7	4	5
<u>Set versus mob stocking</u>	Set	0	4
	equal	10	9
	mob	4	5
<u>Stocking density (per</u>	< 7	0	1
<u>acre) when mob</u>	7 - 15	6	1
<u>stocked</u>	16 - 30	3	1
	> 30	5	11
<u>Major types of livestock</u>	sheep	0	16
<u>farmed</u>	sheep and beef		
	cattle	14	0
	sheep and dairy		
	cattle	0	2

Table XXI Contd

<u>FERTILISERS USED ON FARM</u>		Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
<u>Super phosphate</u> usage	< 10	8	8
(cwt/acre over last seven years)	10 - 25	6	10
<u>Potassic fertiliser</u> usage	Nil	8	16
(cwt/acre over last seven years)	< 4	6	2
$\chi^2 = 6.0 \quad p < .05$			
<u>Lime</u> usage (cwt/acre over last seven years)	Nil	6	7
	< 10	6	9
	> 10	2	2

PASTURE INSECTICIDES

<u>D.D.T.</u> usage (lbs/acre over last seven years)	Nil	8	10
	< 1	1	2
	1 - 2	1	1
	> 2	4	4
Farms where <u>fenitrothion</u> not used		13	17
Farms where fenitrothion used		1	1

ANIMAL INSECTICIDES

Farms not using <u>diazinon</u>	7	7
Farms using diazinon	6	11
Farms not using <u>V.C.13</u>	11	16
Farms using V.C.13	1	2
Farms not using <u>asuntol</u>	11	16
Farms using asuntol	1	2
Farms not using <u>carbophenothion</u>	11	18
Farms using carbophenothion	1	0

Table XXI Contd

<u>Animal Insecticides</u>		Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Farms not using <u>arsenic</u>		8	14
Farms using arsenic		5	4
Farms not using <u>derris</u>		9	14
Farms using derris		4	4
Farms not using <u>sulphur</u>		11	14
Farms using sulphur		2	2
Farms not using <u>cresylic acid</u>		13	16
Farms using cresylic acid		0	2
Farms not using <u>parex</u>		12	17
Farms using parex		1	1

ANTHELMINTICS

Farms not using <u>thiabendazole</u>		10	7
Farms using thiabendazole		4	10
Farms not using <u>nilverm</u>		12	15
Farms using nilverm		2	2
Farms not using <u>N.C.A.</u>		11	14
Farms using N.C.A.		3	3

OTHER ANIMAL REMEDIES

Times <u>Clostridial vaccines</u>	nil	7	3
administered.	once	1	5
	twice	1	0
	more than twice	4	6
<u>Selenium usage</u> on ewes	Nil	8	10
slaughtered	less than annually	2	1
	at least annually	3	1

Table XXI Contd

<u>Other Animal Remedies</u>		Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
Availability of <u>stock licks</u> to sheep	Nil	8	7
	a little	3	1
	most of the time	2	2

HERBICIDES

<u>Tordon</u> not used on farm	10	14
<u>Tordon</u> used on farm	2	1
<u>2.4.D.</u> not used on farm	11	9
<u>2.4.D.</u> used on farm	1	6
<u>2.4.5.T.</u> not used on farm	10	9
<u>2.4.5.T.</u> used on farm	2	7
<u>M.C.P.A. or M.C.P.B.</u> not used on farm	9	12
<u>M.C.P.A. or M.C.P.B.</u> used on farm	3	3
<u>Paraquat</u> not used on farm	9	9
<u>Paraquat</u> used on farm	3	6

DISEASE HISTORY OF SHEEP FLOCK

<u>Facial eczema</u> prevalence	Nil	7	14
	a little	6	3
	much	1	1
Prevalence of <u>Salmonellosis</u>	Nil	7	4
	a little	4	10
	much	3	4

SUPPLEMENTARY FEEDS AVAILABLE

No <u>hay</u> available	1	7
Hay available	4	2

Table XXI Contd

<u>Supplementary Feeds Available</u>	Farms with high prevalence of carcinomas of small intestine	Farms with low prevalence of carcinomas of small intestine
No <u>Chou moellier</u> available	1	3
Chou moellier available	4	6
No <u>root crops</u> available	1	4
Root crops	4	5
No <u>cereal grains</u> available	4	8
Cereal grains available	1	1

(a) Differences Between Farms with High and Low Prevalence
of Small Intestinal Carcinoma

Figure 70 shows the numbers of ewes inspected, and the percentage of those ewes with small intestinal carcinomas, from vendors who had owned these sheep for whole of life, or at least four years. As a working procedure, groups of sheep were selected as having a high tumour prevalence if over 150 ewes were inspected and in excess of 2% were found with carcinomas of the small intestine, or, if in lines of less than 150 ewes, three or more intestinal neoplasms were diagnosed. Low tumour prevalence groups of ewes were considered to be those from which over seventy-five ewes were inspected and in which no neoplasms were found, or from which at least 150 ewes were inspected with carcinomas of the small intestine being diagnosed in less than 1%. The boundaries of these high and low prevalence classifications are shown in Figure 70.

Cross classifications of the 14 high and 18 low tumour prevalence farms with each of the factors covered in the questionnaire (Appendix I) are shown in Table XXI, together with the respective chi squared values and probabilities where $p < .05$. The small number of farms falling into these high and low prevalence classifications (32 farms in all) made it difficult to detect the effects of the various factors. For instance, if the 14 high prevalence farms were evenly divided between presence and absence of a factor, the 18 low prevalence farms would need to be divided 15 : 3 or 3 : 15 for significance at $p < .05$. Factors found by these analyses to be statistically associated with high tumour prevalence were -

T A B L E XXIICattle and Sheep Stocking Rates on High and Low
Tumour Prevalence Farms

Code No.	Area (acres)	Cattle Numbers	Cattle Density (per acre)	Were Cattle mixed with sheep	Sheep Numbers	Sheep (per acre)
Low Tumour Prevalence Farms.						
33	610	-	-	-	1,400	2.295
40	300	150	.500	Yes	1,700	5.666
44	843	150	.178	No	3,556	4.218
70	160	-	-	-	560	3.500
75	633	200	.316	Yes	3,050	4.818
110	230	30	.130	Yes	1,200	5.217
127	1250	320	.256	Yes	5,500	4.400
132	340	75	.221	Yes	1,680	4.941
135	230	70	.304	No	1,100	4.783
139	360	15	.042	Yes	1,140	3.166
149	1516	-	-	-	3,780	2.493
150	1136	220	.194	Yes	4,900	4.313
166	325	57	.175	No	2,000	6.154
170	200	40	.200	No	1,100	5.500
178	3600	200	.055	Yes	6,500	1.805
179	116	30	.259	Yes	600	5.172
201	4000	1303	.326	Yes	7,500	1.875
222	2912	450	.155	Yes	11,000	3.776
Average			.221			4.116
High Tumour Prevalence Farms.						
2	182	30	.165	Yes	1,150	6.319
7	313	100	.319	Yes	1,000	3.195
37	2000	620	.310	Yes	7,000	3.500
46	700	140	.200	Yes	2,700	3.857
88	480	120	.250	Yes	1,510	3.146

Table XXII Contd.

Cattle and Sheep Stocking Rates on High and Low
Tumour Prevalence Farms

Code No.	Area (acres)	Cattle Numbers	Cattle Density (per acre)	Were Cattle mixed with sheep	Sheep Numbers	Sheep (per acre)
107	480	120	.250	Yes	1,870	3.896
120	120	30	.250	Yes	600	5.000
141	1000	300	.300	Yes	2,400	2.400
152	440	-	-	-	3,000	6.818
158	250	40	.160	Yes	900	3.600
159	241	40	.166	Yes	1,600	6.639
194	273	100	.366	Yes	1,350	4.945
203	676	104	.154	Yes	1,904	2.816
215	1259	136	.108	Yes	3,511	2.789
Average			<u>.231</u>			<u>4.209</u>

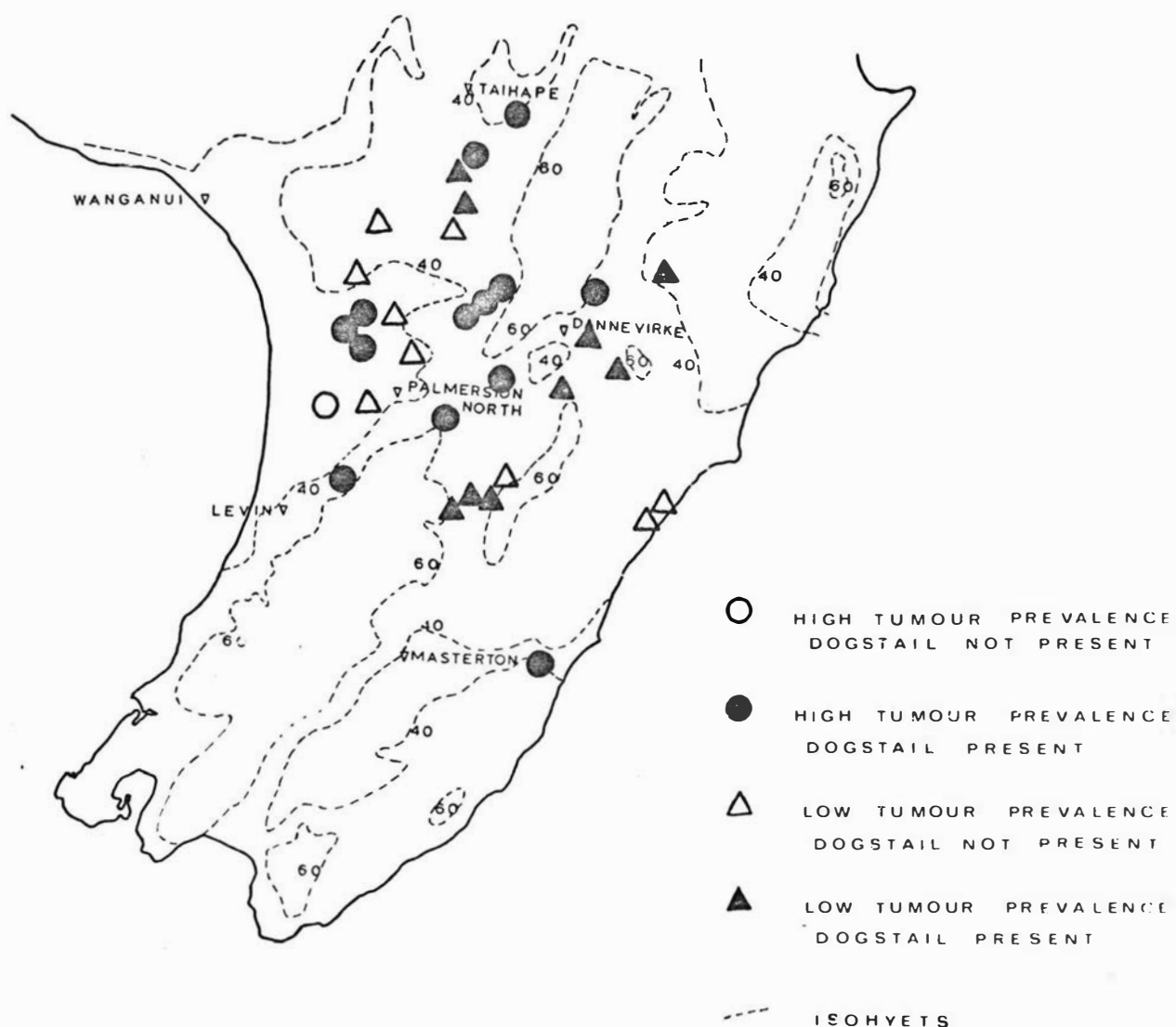


Figure 71

The locations of farms forwarding ewes with high or low prevalence of carcinoma of the small intestine, showing the rainfall status of those farms and whether or not the vendors reported crested dogstail was present on those farms.

- (1) the presence of crested dogstail,
- (2) the greater use of potassic fertilisers,
- and (3) the nomination of farms of origin as "sheep and beef" farms, as opposed to "sheep" or "sheep and dairy" farms.

These classifications were recognised as subjective and all but four of these vendors of high and low prevalence groups were questioned again as to the actual numbers of beef cattle they had been farming during 1967, and whether these cattle were grazed together with ewes in the same paddock. Table XXII lists the average sheep stocking rates and average cattle stocking rates on these farms, together with the farmers' replies as to whether cattle were grazed with the ewes. This failed to reveal any differences between high and low tumour prevalence farms in terms of either average sheep or cattle densities. On all 13 high prevalence farms for which information was available, cattle were grazed mixed with sheep whereas four of the 15 vendors of low prevalence groups of ewes reported they grazed their sheep separately from their cattle. This difference was not statistically significant.

Figure 71 maps the locations of high and low tumour prevalence farms and shows their rainfall status and whether or not crested dogstail was reported on those farms. No rainfall pattern is apparent, however, there is an apparent geographic difference in that nearly all farms mapped east of the North Island main dividing range had a low tumour prevalence.

T A B L E XXIII

Tabulations of Sheep With, and Without, Carcinomas of the
Small Intestine According to Known Exposure to Various
Physiographic, Botanical and Managerial Factors

<u>SOIL TYPES</u>	Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
Sheep not exposed to <u>Soil Type 1</u>	19,356	304	5,744	79	13,642	225
Sheep exposed to <u>Soil Type 1</u>	2,393	38	509	5	1,835	33
	$\chi^2 = 0.000$	N.S.	$\chi^2 = 0.279$	N.S.	$\chi^2 = 0.048$	N.S.
Sheep not exposed to <u>Soil Type 2</u>	19,472	305	6,067	80	13,415	225
Sheep exposed to <u>Soil Type 2</u>	2,373	41	186	4	2,187	37
	$\chi^2 = 0.247$	N.S.	..		$\chi^2 = 0.001$	N.S.
Sheep not exposed to <u>Soil Type 8</u>	21,620	346	6,093	84	15,527	262
Sheep exposed to <u>Soil Type 8</u>	235	0	160	0	75	0
Sheep not exposed to <u>Soil Type 9</u>	21,636	346	6,034	84	15,602	262
Sheep exposed to <u>Soil Type 9</u>	219	0	219	0	0	0
Sheep not exposed to <u>Soil Type 11</u>	21,768	344	6,241	84	15,527	260
Sheep exposed to <u>Soil Type 11</u>	87	2	12	0	75	2
Sheep not exposed to <u>Soil Type 12</u>	20,443	324	5,850	78	14,593	246
Sheep exposed to <u>Soil Type 12</u>	1,412	22	403	6	1,009	16
	$\chi^2 = 0.001$	N.S.			$\chi^2 = 0.011$	N.S.
Sheep not exposed to <u>Soil Type 13</u>	14,589	207	4,575	61	10,014	146
Sheep exposed to <u>Soil Type 13</u>	8,266	139	1,678	23	5,588	116
	$\chi^2 = 2.197$	N.S.	$\chi^2 = 0.000$	N.S.	$\chi^2 = 8.022$	p < .01
Sheep not exposed to <u>Soil Type 18</u>	21,836	346	6,253	84	15,583	262
Sheep exposed to <u>Soil Type 18</u>	19	0	0	0	19	0
Sheep not exposed to <u>Soil Type 23</u>	20,331	316	6,112	81	14,219	235
Sheep exposed to <u>Soil Type 23</u>	1,524	30	141	3	1,383	27
	$\chi^2 = 1.257$	N.S.			$\chi^2 = 0.494$	N.S.
Sheep not exposed to <u>Soil Type 25</u>	21,546	342	5,944	80	15,602	262
Sheep exposed to <u>Soil Type 25</u>	309	4	309	4	0	0
Sheep not exposed to <u>Soil Type 27</u>	21,671	345	6,253	84	15,418	261
Sheep exposed to <u>Soil Type 27</u>	184	1	0	0	184	1
Sheep not exposed to <u>Soil Type 28</u>	21,789	343	6,187	81	15,602	262
Sheep exposed to <u>Soil Type 28</u>	66	3	66	3	0	0
Sheep not exposed to <u>Soil Type 29</u>	20,594	329	5,588	77	15,006	252
Sheep exposed to <u>Soil Type 29</u>	1,261	17	665	7	596	10
	$\chi^2 = 0.316$	N.S.	$\chi^2 = 0.252$	N.S.	$\chi^2 = 0.025$	N.S.
Sheep not exposed to <u>Soil Type 31</u>	21,690	345	6,088	83	15,602	262
Sheep exposed to <u>Soil Type 31</u>	165	1	165	1	0	0
Sheep not exposed to <u>Soil Type 66</u>	21,747	343	6,253	84	15,494	259
Sheep exposed to <u>Soil Type 66</u>	108	3	0	0	108	3
Sheep not exposed to <u>Soil Type 75</u>	20,893	332	6,050	81	14,843	251
Sheep exposed to <u>Soil Type 75</u>	962	14	203	3	759	11
	$\chi^2 = 0.035$	N.S.			$\chi^2 = 0.124$	N.S.
Sheep not exposed to <u>Soil Type 76</u>	19,932	325	5,101	73	14,831	252
Sheep exposed to <u>Soil Type 76</u>	1,923	21	1,152	11	771	10
	$\chi^2 = 2.843$	N.S.	$\chi^2 = 1.234$	N.S.	$\chi^2 = 0.476$	N.S.

Table XXIII Contd.

SOIL TYPES	Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
Sheep not exposed to <u>Soil Type 77</u>	18,439	295	5,241	61	13,198	234
Sheep exposed to <u>Soil Type 77</u>	3,416	51	1,012	23	2,404	28
	$\chi^2 = 0.142$	N.S.	$\chi^2 = 6.806$	$p < .01$	$\chi^2 = 4.068$	$p < .05$
Sheep not exposed to <u>Soil Type 78</u>	20,833	354	6,154	81	14,679	253
Sheep exposed to <u>Soil Type 78</u>	1,022	12	99	3	923	9
	$\chi^2 = 0.863$	N.S.			$\chi^2 = 2.436$	N.S.
Sheep not exposed to <u>Soil Type 79</u>	21,767	346	6,253	84	15,514	262
Sheep exposed to <u>Soil Type 79</u>	88	0	0	0	88	0
Sheep not exposed to <u>Soil Type 106</u>	19,976	325	6,253	84	13,723	241
Sheep exposed to <u>Soil Type 106</u>	1,879	21	0	0	1,879	21
	$\chi^2 = 2.468$	N.S.			$\chi^2 = 3.592$	N.S.
Sheep not exposed to <u>Soil Type 107</u>	21,129	338	6,253	84	15,076	254
Sheep exposed to <u>Soil Type 107</u>	526	8	0	0	526	8
	$\chi^2 = 0.001$	N.S.			$\chi^2 = 0.012$	N.S.
Sheep not exposed to <u>Soil Type 113</u>	21,796	346	6,232	34	15,564	262
Sheep exposed to <u>Soil Type 113</u>	59	0	21	0	38	0
Sheep not exposed to <u>Soil Type 114</u>	20,315	322	5,481	73	14,834	249
Sheep exposed to <u>Soil Type 114</u>	1,540	24	772	11	768	13
	$\chi^2 = 0.000$	N.S.	$\chi^2 = 0.001$	N.S.	$\chi^2 = 0.013$	N.S.
Sheep not exposed to <u>Soil Type 115</u>	20,578	332	5,589	81	14,989	251
Sheep exposed to <u>Soil Type 115</u>	1,277	14	664	3	613	11
	$\chi^2 = 1.693$	N.S.	$\chi^2 = 3.654$	N.S.	$\chi^2 = 0.003$	N.S.
Sheep not exposed to <u>Soil Type 116</u>	21,615	345	6,235	84	15,380	261
Sheep exposed to <u>Soil Type 116</u>	240	1	18	0	220	1
Sheep not exposed to <u>Soil Type 117</u>	21,058	345	5,812	74	15,246	251
Sheep exposed to <u>Soil Type 117</u>	797	21	441	10	356	11
	$\chi^2 = 4.971$	$p < .05$	$\chi^2 = 2.263$	N.S.	$\chi^2 = 3.383$	N.S.
Sheep not exposed to <u>Soil Type 118</u>	21,206	336	6,237	84	14,969	252
Sheep exposed to <u>Soil Type 118</u>	649	10	16	0	633	10
	$\chi^2 = 0.005$	N.S.			$\chi^2 = 0.001$	N.S.
Sheep not exposed to <u>Soil Type 120</u>	21,552	346	5,950	84	15,602	262
Sheep exposed to <u>Soil Type 120</u>	303	0	303	0	0	0
Sheep not exposed to <u>Soil Type 122</u>	20,893	335	5,618	78	15,275	257
Sheep exposed to <u>Soil Type 122</u>	962	11	635	6	327	5
	$\chi^2 = 0.940$	N.S.	$\chi^2 = 0.529$	N.S.		
Sheep not exposed to <u>Soil Type 124</u>	21,109	336	5,875	77	15,234	259
Sheep exposed to <u>Soil Type 124</u>	746	10	376	7	368	3
	$\chi^2 = 0.146$	N.S.				
Sheep not exposed to <u>Soil Type 125</u>	21,691	345	6,089	83	15,602	262
Sheep exposed to <u>Soil Type 125</u>	164	1	164	1	0	0
CLIMATE						
Annual average	< 46	561	10	561	10	0
temperature ($^{\circ}$ F) on	46 - 49.9	0	0	0	0	0
property of origin	50 - 53.9	10,907	173	3,634	50	7,273
	> 54	10,367	163	2,058	24	8,429
		$\chi^2 = 0.151$	N.S.	$\chi^2 = 1.204$	N.S.	$\chi^2 = 0.001$
Average winter minimum	< 32	561	10	561	10	0
temperature ($^{\circ}$ F) on	32 - 35.9	1,036	15	879	12	217
property of origin	36 - 39.9	16,739	268	4,117	58	12,622
	> 40	3,519	53	756	4	2,763
		$\chi^2 = 0.419$	N.S.	$\chi^2 = 4.721$	N.S.	$\chi^2 = 0.112$

Table XXIII Contd

		Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
		Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
<u>CLIMATE</u>							
Average summer maximum	< 65	561	10	561	10	0	0
temperature (°F) on	65 - 68.9	3,168	54	2,074	35	1,094	19
property of origin	69 - 71.9	17,505	276	3,399	39	14,106	237
	> 72	621	6	219	0	402	6
		$\chi^2 = 2.019$	N.S.	$\chi^2 = 1.253$	N.S.	$\chi^2 = 0.103$	N.S.
Average annual	< 40	8,790	183	1,790	27	8,000	156
rainfall (inches) on	41 - 60	10,377	144	3,634	49	6,743	95
property of origin	61 - 80	1,651	19	792	8	859	11
	> 81	37	0	37	0	0	0
		$\chi^2 = 9.647$	$p < .01$	$\chi^2 = 1.233$	N.S.	$\chi^2 = 7.123$	$p < .05$
<u>ALTITUDE and LATITUDE</u>							
Mean altitude (feet	< 200	3,719	62	363	4	3,356	58
above sea level) of	200 - 499	8,013	137	2,093	33	5,920	104
property or origin	500 - 1999	6,246	83	1,648	11	4,598	72
	1000 - 1999	3,862	64	2,134	36	1,728	28
	> 2000	15	0	15	0	0	0
		$\chi^2 = 3.588$	N.S.	$\chi^2 = 7.778$	$p < .05$	$\chi^2 = 0.636$	N.S.
Latitude (degrees	< 39° 30'	99	3	99	3	0	0
south) of property	39° 30' - 40°	3,054	45	1,401	18	1,653	27
of origin	40° - 40° 30'	15,969	261	3,588	51	12,381	210
	40° 30' - 41°	2,554	34	1,165	12	1,389	22
	> 41°	179	3	0	0	179	3
		$\chi^2 = 0.841$	N.S.	$\chi^2 = 1.038$	N.S.	$\chi^2 = 0.034$	N.S.
<u>PASTURE SPECIES</u>							
Sheep not exposed to ryegrass		395	4	171	3	224	1
Sheep exposed to ryegrass		21,460	342	6,082	81	15,378	261
Sheep not exposed to cocksfoot		9,674	149	2,586	42	7,088	107
Sheep exposed to cocksfoot		12,181	197	3,667	42	8,514	155
		$\chi^2 = 0.153$	N.S.	$\chi^2 = 2.207$	N.S.	$\chi^2 = 2.009$	N.S.
Sheep not exposed to danthonia		17,389	289	4,156	63	13,233	226
Sheep exposed to danthonia		4,466	57	2,097	21	2,369	36
		$\chi^2 = 3.054$	N.S.	$\chi^2 = 2.343$	N.S.	$\chi^2 = 0.312$	N.S.
Sheep not exposed to browntop		11,254	188	1,723	23	9,529	165
Sheep exposed to browntop		10,601	158	4,528	61	6,073	97
		$\chi^2 = 0.990$	N.S.	$\chi^2 = 0.006$	N.S.	$\chi^2 = 0.316$	N.S.
Sheep not exposed to dogstail		8,353	114	2,208	22	6,145	92
Sheep exposed to dogstail		13,502	232	4,045	62	9,457	170
		$\chi^2 = 3.792$	$p < .05$	$\chi^2 = 2.636$	N.S.	$\chi^2 = 1.795$	N.S.
Sheep not exposed to white clover		42	0	5	0	37	0
Sheep exposed to white clover		21,813	346	6,248	84	15,565	262
Sheep not exposed to red clover		17,876	292	4,858	68	13,018	124
Sheep exposed to red clover		3,979	54	1,395	16	2,584	38
		$\chi^2 = 1.378$	N.S.	$\chi^2 = 0.338$	N.S.	$\chi^2 = 0.649$	N.S.
Sheep not exposed to subterranean clover		10,713	174	2,693	35	8,020	139
Sheep exposed to subterranean clover		11,142	172	3,560	49	7,582	123
		$\chi^2 = 0.172$	N.S.	$\chi^2 = 0.021$	N.S.	$\chi^2 = 1.218$	N.S.
Sheep not exposed to Lotus major		18,391	302	4,538	63	13,853	239
Sheep exposed to Lotus major		3,464	44	1,715	21	1,749	23
		$\chi^2 = 2.283$	N.S.	$\chi^2 = 0.138$	N.S.	$\chi^2 = 1.300$	N.S.

Table XXIII Contd

WEED SPECIES		Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
		Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
Prevalence of <u>bracken</u>	Not present	15,958	250	3,322	42	12,636	208
<u>fern</u> on property of	Uncommon	3,894	52	1,974	22	1,920	30
origin	Common	1,927	41	891	17	1,036	24
		$\chi^2 = 5.067$	N.S.	$\chi^2 = 3.010$	N.S.	$\chi^2 = 2.682$	N.S.
Prevalence of <u>other</u>	Not present	19,223	304	4,716	63	14,507	241
<u>ferns</u> on property	Uncommon	2,085	34	1,320	18	765	16
of origin	Common	481	5	151	0	330	5
		$\chi^2 = 0.021$	N.S.	$\chi^2 = 0.038$	N.S.	$\chi^2 = 0.253$	N.S.
Prevalence of <u>ragwort</u>	Not present	11,972	180	2,250	38	9,722	142
on property of origin	Uncommon	8,617	132	3,347	37	5,270	95
	Common	1,200	21	590	6	610	15
		$\chi^2 = 0.427$	N.S.	$\chi^2 = 3.867$	N.S.	$\chi^2 = 5.168$	N.S.
Sheep not exposed to <u>barberry</u>		19,359	313	5,762	76	13,797	237
Sheep exposed to barberry		2,296	33	491	8	1,805	25
		$\chi^2 = 0.244$	N.S.	$\chi^2 = 0.130$	N.S.	$\chi^2 = 0.848$	N.S.
Sheep not exposed to <u>barley grass</u>		6,131	106	2,448	37	3,683	69
Sheep exposed to barley grass		15,724	240	3,805	47	11,919	193
		$\chi^2 = 1.000$	N.S.	$\chi^2 = 0.641$	N.S.	$\chi^2 = 0.917$	N.S.
Sheep not exposed to <u>blackberry</u>		18,313	291	5,215	70	13,098	221
Sheep exposed to blackberry		3,542	55	1,038	14	2,504	41
		$\chi^2 = 0.006$	N.S.	$\chi^2 = 0.017$	N.S.	$\chi^2 = 0.308$	N.S.
Sheep not exposed to <u>broom</u>		21,133	336	5,977	82	15,156	254
Sheep exposed to broom		722	10	276	2	446	8
		$\chi^2 = 0.075$	N.S.			$\chi^2 = 0.000$	N.S.
Sheep not exposed to <u>buttercup</u>		17,959	292	5,476	76	12,483	216
Sheep exposed to buttercup		3,896	54	777	8	3,119	46
		$\chi^2 = 1.000$	N.S.	$\chi^2 = 0.403$	N.S.	$\chi^2 = 0.809$	N.S.
Sheep not exposed to <u>capeweed</u>		21,493	343	6,253	84	15,240	259
Sheep exposed to capeweed		362	3	0	0	362	3
Sheep not exposed to <u>chickweed</u>		20,937	332	5,960	84	14,977	248
Sheep exposed to chickweed		918	14	293	0	625	14
		$\chi^2 = 0.000$	N.S.			$\chi^2 = 0.871$	N.S.
Sheep not exposed to <u>couch</u>		18,626	300	5,417	72	13,209	228
Sheep exposed to couch		3,229	46	836	12	2,393	34
		$\chi^2 = 0.481$	N.S.	$\chi^2 = 0.007$	N.S.	$\chi^2 = 0.933$	N.S.
Sheep not exposed to <u>dandelion</u>		9,151	165	2,455	35	6,696	130
Sheep exposed to dandelion		12,704	181	3,798	49	8,906	132
		$\chi^2 = 4.495$	$p < .05$	$\chi^2 = 0.112$	N.S.	$\chi^2 = 4.450$	$p < .05$
Sheep not exposed to <u>daisy</u>		15,044	248	4,157	63	10,887	185
Sheep exposed to daisy		6,811	98	2,036	21	4,715	77
		$\chi^2 = 1.153$	N.S.	$\chi^2 = 2.335$	N.S.	$\chi^2 = 0.049$	N.S.
Sheep not exposed to <u>dog daisy</u>		21,508	337	6,241	84	15,267	253
Sheep exposed to dog daisy		347	9	12	0	335	9
		$\chi^2 = 1.621$	N.S.			$\chi^2 = 1.453$	N.S.
Sheep not exposed to <u>dock</u>		13,442	217	4,020	55	9,422	162
Sheep exposed to dock		8,413	129	2,233	29	6,180	100
		$\chi^2 = 0.163$	N.S.	$\chi^2 = 0.012$	N.S.	$\chi^2 = 0.167$	N.S.
Sheep not exposed to <u>fat hen</u>		15,169	245	4,471	68	10,698	177
Sheep exposed to fat hen		6,686	101	1,782	16	4,904	85
		$\chi^2 = 0.252$	N.S.	$\chi^2 = 3.192$	N.S.	$\chi^2 = 0.079$	N.S.
Sheep not exposed to <u>fennel</u>		21,367	331	6,131	83	15,236	248
Sheep exposed to fennel		488	15	122	1	366	14
		$\chi^2 = 5.882$	$p < .05$			$\chi^2 = 8.662$	$p < .01$

Table XXIII Contd

WEED SPECIES	Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
Sheep not exposed to <u>fireweed</u>	21,706	343	6,104	81	15,602	262
Sheep exposed to fireweed	149	3	149	3	0	0
Sheep not exposed to <u>foxglove</u>	20,319	325	5,132	71	15,187	254
Sheep exposed to foxglove	1,536	21	1,121	13	415	8
	$\chi^2 = 0.344$	N.S.	$\chi^2 = 0.192$	N.S.	$\chi^2 = 0.039$	N.S.
Sheep not exposed to <u>gorts rue</u>	21,156	234	6,253	84	14,903	250
Sheep exposed to gorts rue	699	12	0	0	699	12
	$\chi^2 = 1.697$	N.S.			$\chi^2 = 0.005$	N.S.
Sheep not exposed to <u>gorse</u>	18,158	281	4,886	66	13,272	215
Sheep exposed to gorse	3,697	65	1,367	18	2,330	47
	$\chi^2 = 0.718$	N.S.	$\chi^2 = 0.001$	N.S.	$\chi^2 = 1.598$	N.S.
Sheep not exposed to <u>hawk weed</u>	19,874	318	5,338	75	14,536	243
Sheep exposed to hawk weed	1,981	28	915	9	1,066	19
	$\chi^2 = 0.281$	N.S.	$\chi^2 = 0.731$	N.S.	$\chi^2 = 0.020$	N.S.
Sheep not exposed to <u>hemlock</u>	17,601	275	5,047	67	12,554	208
Sheep exposed to hemlock	4,254	71	1,206	17	3,048	54
	$\chi^2 = 0.179$	N.S.	$\chi^2 = 0.006$	N.S.	$\chi^2 = 0.127$	N.S.
Sheep not exposed to <u>lambs tongue</u>	21,588	344	6,093	84	15,495	260
Sheep exposed to lambs tongue	267	2	160	0	107	2
Sheep not exposed to <u>linseed</u>	21,589	338	6,187	81	15,402	257
Sheep exposed to linseed	266	8	66	3	200	5
	$\chi^2 = 2.512$	N.S.				
Sheep not exposed to <u>lupin</u>	20,674	327	5,913	78	14,761	249
Sheep exposed to lupin	1,181	19	340	6	841	13
	$\chi^2 = 0.002$	N.S.			$\chi^2 = 0.027$	N.S.
Sheep not exposed to <u>native bush</u>	17,942	282	4,436	54	13,506	228
Sheep exposed to native bush	3,913	64	1,817	30	2,096	34
	$\chi^2 = 0.046$	N.S.	$\chi^2 = 1.470$	N.S.	$\chi^2 = 0.015$	N.S.
Sheep not exposed to <u>black nightshade</u>	17,672	278	5,206	73	12,466	205
Sheep exposed to black nightshade	4,183	68	1,047	11	3,136	57
	$\chi^2 = 0.029$	N.S.	$\chi^2 = 0.552$	N.S.	$\chi^2 = 0.342$	N.S.
Sheep not exposed to <u>penny royal</u>	13,402	207	2,956	42	10,446	165
Sheep exposed to penny royal	8,453	139	3,297	42	5,156	97
	$\chi^2 = 0.261$	N.S.	$\chi^2 = 0.149$	N.S.	$\chi^2 = 1.663$	N.S.
Sheep not exposed to <u>plantain</u>	18,468	296	5,788	82	12,680	214
Sheep exposed to plantain	3,387	50	465	2	2,922	48
	$\chi^2 = 0.210$	N.S.			$\chi^2 = 0.007$	N.S.
Sheep not exposed to <u>prickly apple</u>	21,426	336	6,050	81	15,376	255
Sheep exposed to prickly apple	429	10	203	3	226	7
	$\chi^2 = 1.070$	N.S.				
Sheep not exposed to <u>rats tail</u>	20,327	327	5,505	79	14,822	248
Sheep exposed to rats tail	1,528	19	748	5	780	14
	$\chi^2 = 0.962$	N.S.	$\chi^2 = 2.314$	N.S.	$\chi^2 = 0.012$	N.S.
Sheep not exposed to <u>red root</u>	20,747	325	5,969	81	14,778	244
Sheep exposed to red root	1,108	21	284	3	824	18
	$\chi^2 = 0.513$	N.S.			$\chi^2 = 0.997$	N.S.
Sheep not exposed to <u>rushes</u>	13,364	224	2,458	32	10,906	192
Sheep exposed to rushes	8,491	122	3,795	52	4,696	70
	$\chi^2 = 1.702$	N.S.	$\chi^2 = 0.012$	N.S.	$\chi^2 = 1.245$	N.S.
Sheep not exposed to <u>sedge</u>	21,748	344	6,253	84	15,495	260
Sheep exposed to sedge	107	2	0	0	107	2

Table XXIII Contd

WEED SPECIES	Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
Sheep not exposed to <u>spurry</u>	19,323	306	6,050	82	13,273	224
Sheep exposed to spurry	2,532	40	203	2	2,329	38
	$\chi^2 = 0.004$	N.S.			$\chi^2 = 0.010$	N.S.
Sheep not exposed to <u>stinging nettle</u>	15,568	271	4,082	59	11,486	212
Sheep exposed to stinging nettle	6,287	75	2,171	25	4,116	50
	$\chi^2 = 8.032$	$p < .01$	$\chi^2 = 0.693$	N.S.	$\chi^2 = 6.731$	$p < .01$
Sheep not exposed to <u>storks bill</u>	21,643	343	6,081	83	15,562	260
Sheep exposed to storks bill	212	3	172	1	40	2
Sheep not exposed to <u>tall fescue</u>	20,601	329	5,872	82	14,729	247
Sheep exposed to tall fescue	1,254	17	381	2	873	15
	$\chi^2 = 0.289$	N.S.			$\chi^2 = 0.002$	N.S.
Sheep not exposed to <u>californian thistle</u>	7,735	144	1,641	20	6,094	124
Sheep exposed to californian thistle	14,120	202	4,612	64	9,508	138
	$\chi^2 = 5.498$	$p < .05$	$\chi^2 = 0.143$	N.S.	$\chi^2 = 7.050$	$p < .01$
Sheep not exposed to <u>scotch thistle</u>	5,335	96	1,638	25	3,677	71
Sheep exposed to scotch thistle	16,520	250	4,595	59	11,925	191
	$\chi^2 = 1.873$	N.S.	$\chi^2 = 0.296$	N.S.	$\chi^2 = 1.590$	N.S.
Sheep not exposed to <u>swamp thistle</u>	19,724	313	5,578	71	14,146	242
Sheep exposed to swamp thistle	2,131	33	675	13	1,456	20
	$\chi^2 = 0.001$	N.S.	$\chi^2 = 1.424$	N.S.	$\chi^2 = 0.691$	N.S.
Sheep not exposed to <u>variegated thistle</u>	18,743	302	4,534	66	14,209	236
Sheep exposed to variegated thistle	3,112	44	1,719	18	1,393	26
	$\chi^2 = 0.528$	N.S.	$\chi^2 = 1.241$	N.S.	$\chi^2 = 0.203$	N.S.
Sheep not exposed to <u>wing thistle</u>	15,878	260	4,255	55	11,623	205
Sheep exposed to wing thistle	5,977	86	1,998	29	3,979	57
	$\chi^2 = 0.944$	N.S.	$\chi^2 = 0.147$	N.S.	$\chi^2 = 1.715$	N.S.
Sheep not exposed to <u>trefoil</u>	18,715	309	4,859	69	13,856	240
Sheep exposed to trefoil	3,140	37	1,394	15	1,746	22
	$\chi^2 = 3.455$	N.S.	$\chi^2 = 0.704$	N.S.	$\chi^2 = 1.758$	N.S.
Sheep not exposed to <u>water cress</u>	19,645	327	5,147	73	14,498	251
Sheep exposed to water cress	2,210	19	1,106	8	1,104	11
	$\chi^2 = 7.548$	$p < .01$	$\chi^2 = 3.269$	N.S.	$\chi^2 = 2.839$	N.S.
Sheep not exposed to <u>wild carrot</u>	21,455	341	6,013	81	15,442	260
Sheep exposed to wild carrot	400	5	240	3	160	2
Sheep not exposed to <u>wild turnip</u>	21,076	337	5,690	80	15,386	257
Sheep exposed to wild turnip	779	9	563	4	216	5
	$\chi^2 = 0.663$	N.S.	$\chi^2 = 1.346$	N.S.		
Sheep not exposed to <u>willow weed</u>	16,239	250	4,809	68	11,430	182
Sheep exposed to willow weed	5,596	96	1,444	16	4,152	80
	$\chi^2 = 0.710$	N.S.	$\chi^2 = 0.553$	N.S.	$\chi^2 = 1.831$	N.S.
Sheep not exposed to <u>wire weed</u>	21,669	342	6,147	81	15,522	261
Sheep exposed to wire weed	186	4	106	3	80	1
Sheep not exposed to <u>yarrow</u>	19,684	318	5,128	71	14,556	247
Sheep exposed to yarrow	2,171	28	1,125	13	1,046	15
	$\chi^2 = 1.095$	N.S.	$\chi^2 = 0.205$	N.S.	$\chi^2 = 0.254$	N.S.
Sheep not exposed to <u>yorkshire fog</u>	17,501	292	4,836	77	12,665	219
Sheep exposed to yorkshire fog	4,354	54	1,417	11	2,937	43
	$\chi^2 = 3.719$	N.S.	$\chi^2 = 3.814$	$p < .05$	$\chi^2 = 0.831$	N.S.
Sheep not exposed to <u>land cress</u>	21,496	335	6,242	84	15,254	251
Sheep exposed to land cress	359	11	11	0	348	11
	$\chi^2 = 4.014$	$p < .05$			$\chi^2 = 3.666$	N.S.

Table XXIII Contd

FEED SPECIES	Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
Sheep not exposed to <u>mallo</u>	21,471	340	6,082	84	15,389	256
Sheep exposed to <u>mallo</u>	384	6	171	0	213	6
Sheep not exposed to <u>nodding</u> <u>thistle</u>	21,490	341	6,050	81	15,440	260
Sheep exposed to <u>nodding</u> <u>thistle</u>	365	5	203	3	162	2
Sheep not exposed to <u>burr dock</u>	21,557	344	6,211	84	15,346	260
Sheep exposed to <u>burr dock</u>	298	2	42	0	256	2
Sheep not exposed to <u>ink weed</u>	20,829	330	5,852	80	14,967	250
Sheep exposed to <u>ink weed</u>	1,036	16	401	4	635	12
	$\chi^2 = 0.000$	N.S.			$\chi^2 = 0.065$	N.S.
Sheep not exposed to <u>shepherds</u> <u>purse</u>	21,789	343	6,187	81	15,602	262
Sheep exposed to <u>shepherds</u> <u>purse</u>	66	3	66	3	0	0
STOCK MANAGEMENT						
Sheep breeds - <u>romney</u>	19,913	321	5,315	70	14,598	251
romney cross	1,849	23	897	14	952	9
other British breeds	45	2	41	0	52	2
	$\chi^2 = 0.966$	N.S.	$\chi^2 = 0.073$	N.S.	$\chi^2 = 1.795$	N.S.
Age of sheep slaughtered (years)						
< 5	594	6	515	6	70	0
5	3,107	46	1,562	21	1,545	25
6	10,903	160	3,287	41	7,616	119
7	6,164	118	783	12	5,381	106
> 7	1,046	15	65	3	981	12
	$\chi^2 = 6.672$	N.S.	$\chi^2 = 1.471$	N.S.	$\chi^2 = 4.649$	N.S.
Reason sheep slaughtered -						
Age	1,942	38	389	6	1,553	32
Tooth conditions	17,160	272	4,863	69	12,297	203
Poor condition	1,899	28	274	3	1,625	25
Infertility	407	4	343	2	64	2
Visceral disease	278	0	215	0	63	0
Surplus to requirements	169	4	169	4	0	0
	$\chi^2 = 1.701$	N.S.			$\chi^2 = 1.563$	N.S.
Size of farm (acres)						
< 100	1,619	27	0	0	1,619	27
100 - 499	14,943	237	3,147	50	11,796	187
500 - 1499	4,158	71	1,971	23	2,187	48
> 1500	1,135	11	1,135	11	0	0
	$\chi^2 = 3.149$	N.S.	$\chi^2 = 3.012$	N.S.	$\chi^2 = 3.998$	N.S.
Number of ewes farmed						
< 200	862	15	0	0	862	15
200 - 499	1,067	8	95	0	972	8
500 - 1499	12,773	217	3,325	50	9,448	167
1500 - 4999	7,098	106	2,778	34	4,320	72
> 5000	55	0	55	0	0	0
	$\chi^2 = 6.297$	N.S.	$\chi^2 = 0.597$	N.S.	$\chi^2 = 4.662$	N.S.
Average sheep stocking rate (per acre)						
< 2	679	5	299	0	380	5
2 - 3	1,527	21	789	10	738	11
3 - 5	10,235	171	3,628	50	6,607	121
5 - 7	8,402	132	1,537	24	6,865	108
> 7	1,012	17	0	0	1,012	17
	$\chi^2 = 4.018$	N.S.	$\chi^2 = 2.012$	N.S.	$\chi^2 = 1.751$	N.S.
Set versus mob stocking						
set	2,454	27	836	2	1,628	25
equal	12,168	205	3,720	56	8,448	149
mob	7,223	114	1,697	26	5,526	88
	$\chi^2 = 4.438$	N.S.	$\chi^2 = 8.727$	$p < .05$	$\chi^2 = 0.793$	N.S.

Table XXIII Contd

		Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
<u>STOCK MANAGEMENT</u>		Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
<u>Stocking density</u> (per acre) < 7		399	2	274	0	125	2
when mob stocked 7 - 15		3,197	61	1,514	30	1,683	31
16 - 30		6,141	100	2,109	32	4,032	64
> 30		9,654	156	1,520	20	8,134	136
		$\chi^2 = 0.504$	N.S.	$\chi^2 = 0.701$	N.S.	$\chi^2 = 0.204$	N.S.
<u>Major types of livestock</u>							
<u>farmed</u> - sheep		2,064	39	275	3	1,789	36
sheep & beef cattle		18,323	290	5,611	80	12,712	210
beef cattle		0	0	0	0	0	0
dairy cattle		24	1	0	0	24	1
sheep & dairy cattle		1,444	16	367	1	1,077	15
		$\chi^2 = 3.242$	N.S.			$\chi^2 = 1.735$	N.S.
<u>FERTILISERS USED ON FARM</u>							
<u>Super phosphate usage</u> Nil		269	5	33	0	236	5
(cwt/acre over last seven years) < 10		10,335	172	3,389	52	6,946	120
10 - 25		10,764	165	2,831	32	7,933	133
25 - 50		127	0	0	0	127	0
> 50		0	0	0	0	0	0
		$\chi^2 = 0.817$	N.S.	$\chi^2 = 1.447$	N.S.	$\chi^2 = 2.420$	N.S.
<u>Potassic fertiliser</u> Nil		11,520	183	4,058	48	7,462	135
usage (cwt/acre over last seven years) < 4		8,644	145	2,065	36	6,579	109
> 4		911	10	130	0	781	10
		$\chi^2 = 1.743$	N.S.	$\chi^2 = 1.857$	N.S.	$\chi^2 = 1.356$	N.S.
<u>Lime usage</u> (cwt/acre Nil		8,307	145	2,653	33	5,654	107
over last seven years) < 10		9,020	130	3,188	41	5,832	89
> 10		4,022	61	272	5	3,750	56
		$\chi^2 = 2.604$	N.S.	$\chi^2 = 0.051$	N.S.	$\chi^2 = 3.502$	N.S.
<u>PASTURE INSECTICIDES</u>							
<u>D.D.T. usage</u> (lbs/acre Nil		10,597	166	3,213	42	7,384	124
over last seven years) < 1		2,069	29	641	7	1,428	22
1 - 2		4,733	80	1,010	16	3,723	64
> 2		3,857	58	1,225	18	2,632	40
		$\chi^2 = 0.904$	N.S.	$\chi^2 = 0.870$	N.S.	$\chi^2 = 0.512$	N.S.
Sheep not exposed to fenitrothion		21,458	339	5,991	82	15,467	257
Sheep exposed to fenitrothion		397	7	262	2	135	5
Sheep not exposed to dioxinon		21,837	346	6,235	84	15,602	262
Sheep exposed to dioxinon		18	0	18	0	0	0
Sheep not exposed to metacystox		21,770	345	6,168	83	15,602	262
Sheep exposed to metacystox		85	1	85	1	0	0
<u>ANIMAL INSECTICIDES</u>							
Sheep not exposed to dioxinon		10,708	175	2,469	35	8,239	140
Sheep exposed to dioxinon		9,255	133	3,568	45	5,687	88
		$\chi^2 = 1.108$	N.S.	$\chi^2 = 0.160$	N.S.	$\chi^2 = 0.378$	N.S.
Sheep not exposed to lucijet		19,462	319	5,724	74	13,738	225
Sheep exposed to lucijet		476	8	313	6	163	2
		$\chi^2 = 0.012$	N.S.				
Sheep not exposed to V.C.13		17,844	279	4,897	64	12,947	215
Sheep exposed to V.C.13		2,094	28	1,140	16	954	12
		$\chi^2 = 0.476$	N.S.	$\chi^2 = 0.012$	N.S.	$\chi^2 = 0.641$	N.S.
Sheep not exposed to asuntol		18,874	291	5,630	73	13,244	218
Sheep exposed to asuntol		1,064	16	407	7	657	9
		$\chi^2 = 0.000$	N.S.			$\chi^2 = 0.143$	N.S.
Sheep not exposed to carbophenothion		19,503	292	6,027	80	13,476	212
Sheep exposed to carbophenothion		435	15	10	0	425	15
		$\chi^2 = 8.96$	p < .01			$\chi^2 = 8.192$	p < .01

Table XXIII Contd.

	Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
<u>ANIMAL INSECTICIDES</u>						
Sheep not exposed to <u>arsenic</u>	12,261	188	4,184	52	8,077	136
Sheep exposed to arsenic	7,831	123	2,007	32	5,824	91
	$\chi^2 = 0.021$	N.S.	$\chi^2 = 0.972$	N.S.	$\chi^2 = 0.230$	N.S.
Sheep not exposed to <u>derris</u>	12,788	200	4,411	56	8,377	144
Sheep exposed to derris	7,238	108	1,714	25	5,524	83
	$\chi^2 = 0.109$	N.S.	$\chi^2 = 0.201$	N.S.	$\chi^2 = 0.812$	N.S.
Sheep not exposed to <u>sulphur</u>	17,305	266	5,257	68	12,189	198
Sheep exposed to sulphur	2,633	41	780	12	1,712	29
	$\chi^2 = 0.000$	N.S.	$\chi^2 = 0.146$	N.S.	$\chi^2 = 0.011$	N.S.
Sheep not exposed to <u>creosylic acid</u>	15,972	251	5,391	74	10,581	177
Sheep exposed to creosylic acid	4,054	57	734	7	3,320	50
	$\chi^2 = 0.464$	N.S.	$\chi^2 = 0.560$	N.S.	$\chi^2 = 0.327$	N.S.
Sheep not exposed to <u>parex</u>	19,502	302	5,601	75	13,901	227
Sheep exposed to parex	656	13	436	5	220	8
	$\chi^2 = 0.496$	N.S.			$\chi^2 = 3.929$	$p < .05$
<u>ANTHELMINTICS</u>						
Sheep not exposed to <u>phenothiazine</u>	21,226	340	5,708	80	15,518	260
Sheep exposed to phenothiazine	394	4	394	4	0	0
Sheep not exposed to <u>thiabendazole</u>	13,782	226	1,416	16	12,366	210
Sheep exposed to thiabendazole	7,838	118	4,686	68	3,152	50
	$\chi^2 = 0.476$	N.S.	$\chi^2 = 0.588$	N.S.	$\chi^2 = 0.123$	N.S.
Sheep not exposed to <u>nilverm</u>	20,039	322	5,106	71	14,933	251
Sheep exposed to nilverm	1,581	22	996	13	585	9
	$\chi^2 = 0.296$	N.S.	$\chi^2 = 0.003$	N.S.	$\chi^2 = 0.008$	N.S.
Sheep not exposed to <u>mintic</u>	21,594	344	6,076	84	15,518	260
Sheep exposed to mintic	26	0	26	0	0	0
Sheep not exposed to <u>N.C.A.</u>	18,669	299	4,473	60	14,196	239
Sheep exposed to N.C.A.	2,950	45	1,629	24	1,322	21
	$\chi^2 = 0.096$	N.S.	$\chi^2 = 0.068$	N.S.	$\chi^2 = 0.019$	N.S.
Sheep not exposed to <u>rametin</u>	21,373	342	6,035	84	15,338	258
Sheep exposed to rametin	247	2	67	0	180	2
Sheep not exposed to <u>loxon</u>	21,456	343	5,938	83	15,518	260
Sheep exposed to loxon	164	1	164	1	0	0
<u>OTHER ANIMAL REMEDIES</u>						
Times <u>clostridial vaccine</u> Nil	3,019	43	1,976	35	1,043	8
administered once	3,566	53	1,507	12	2,059	41
"twice	1,913	34	515	9	1,398	25
more than twice	4,384	64	2,199	28	2,185	36
	$\chi^2 = 1.120$	N.S.	$\chi^2 = 6.637$	N.S.	$\chi^2 = 6.491$	N.S.
<u>Selenium usage</u> on ewes Nil	5,843	76	4,715	57	1,128	19
slaughtered, less than annually	1,444	23	1,057	19	387	4
at least annually	1,850	30	481	8	1,369	22
	$\chi^2 = 1.401$	N.S.	$\chi^2 = 2.581$	N.S.	$\chi^2 = 0.073$	N.S.
Availability of <u>stock</u> Nil	4,754	57	3,804	47	950	10
licks to sheep a little	4,416	77	1,456	23	2,960	54
most of the time	1,829	27	750	14	1,079	13
	$\chi^2 = 4.471$	N.S.	$\chi^2 = 2.205$	N.S.	$\chi^2 = 3.569$	N.S.
<u>HERBICIDES</u>						
Sheep not exposed to <u>tordon</u>	17,651	279	4,166	54	13,485	225
Sheep exposed to tordon	2,129	37	950	14	1,179	23
	$\chi^2 = 0.199$	N.S.	$\chi^2 = 0.071$	N.S.	$\chi^2 = 0.348$	N.S.

Table XXIII contd.

HERBICIDES	Total of all Sheep inspected		Sheep whose whole life history is known		Sheep whose history is known for less than whole of life	
	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours	Sheep without tumours	Sheep with tumours
Sheep not exposed to <u>2.4.D.</u>	15,619	263	3,838	58	11,824	205
Sheep exposed to 2.4.D.	4,141	5*	1,278	10	2,840	43
	$\chi^2 = 3.046$	N.S.	$\chi^2 = 3.263$	N.S.	$\chi^2 = 0.519$	N.S.
Sheep not exposed to <u>2.4.5.T</u>	12,044	195	2,795	43	9,249	152
Sheep exposed to 2.4.5.T	7,870	121	2,455	25	5,415	96
	$\chi^2 = 0.148$	N.S.	$\chi^2 = 2.309$	N.S.	$\chi^2 = 0.260$	N.S.
Sheep not exposed to <u>M.C.P.A.</u> or <u>M.C.P.B.</u>	15,414	240	4,049	51	11,365	189
Sheep exposed to M.C.P.A. or M.C.P.B.	4,366	76	1,067	17	3,299	59
	$\chi^2 = 0.596$	N.S.	$\chi^2 = 0.468$	N.S.	$\chi^2 = 0.165$	N.S.
Sheep not exposed to <u>paraquat</u>	14,066	226	2,913	37	11,153	189
Sheep exposed to paraquat	5,746	90	2,203	31	3,543	59
	$\chi^2 = 0.019$	N.S.	$\chi^2 = 0.086$	N.S.	$\chi^2 = 0.001$	N.S.
DISEASE HISTORY OF SHEEP FLOCK						
<u>Facial eczema prevalence</u>						
Nil	10,126	148	2,917	36	7,209	112
a little	8,790	151	2,352	35	6,438	116
much	2,939	47	984	13	1,955	34
	$\chi^2 = 1.926$	N.S.	$\chi^2 = 0.620$	N.S.	$\chi^2 = 1.273$	N.S.
<u>Prevalence of salmonellosis</u>						
Nil	9,418	141	2,194	36	6,224	105
a little	9,276	141	2,379	27	6,897	114
much	4,161	64	1,680	21	2,481	43
	$\chi^2 = 0.723$	N.S.	$\chi^2 = 2.446$	N.S.	$\chi^2 = 0.072$	N.S.
SUPPLEMENTARY FEEDS AVAILABLE TO SHEEP						
No hay available	5,369	82	2,042	20	3,327	62
Hay available	5,826	101	1,390	21	4,436	80
	$\chi^2 = 0.595$	N.S.	$\chi^2 = 1.510$	N.S.	$\chi^2 = 0.011$	N.S.
No chow moellier available	6,243	102	969	10	5,274	92
Chow moellier available	4,952	81	2,463	31	2,489	50
	$\chi^2 = 0.004$	N.S.	$\chi^2 = 0.136$	N.S.	$\chi^2 = 0.497$	N.S.
No root crops fed	6,598	104	2,174	22	4,424	82
Root crops fed	4,597	79	1,258	19	3,339	60
	$\chi^2 = 0.435$	N.S.	$\chi^2 = 1.245$	N.S.	$\chi^2 = 0.009$	N.S.
No cereal grains fed	10,146	161	3,194	37	6,952	124
Cereal grains fed	1,049	22	238	4	811	18
	$\chi^2 = 1.190$	N.S.			$\chi^2 = 0.519$	N.S.
LENGTH OF TIME SHEEP WERE ON PROPERTY FROM WHICH THEY WERE SLAUGHTERED						
Whole of life	6,253	84				
4 years or more	3,830	51				
Less than 4 years	11,772	211				
	$\chi^2 = 7.231$	$p < .05$				

* N.S. = not significant ($p > .05$)

** Chi squared not calculated where less than 500 sheep without tumours and less than seven sheep with tumours were either exposed or not exposed to a particular factor.

figures joined by a vertical bar were added together for the purposes of chi squared analysis.

Figures indicated by this sign were omitted from the chi squared analysis.

T A B L E XXIV

Prevalence (%) of Carcinomas of the Small Intestine
in Sheep Exposed, and not Exposed to Factors with which
Statistically Significant ($p < .05$) Differences in
Prevalence were Associated

<u>SOIL TYPES</u>	Prevalence (%) of sheep with small intestinal carcinomas		
	Total of all sheep inspected	Sheep whose whole life history was known	Sheep whose history was known for less than whole of life
Sheep not exposed to			
<u>Soil type 13</u>	1.39	1.31	1.43
Sheep exposed to			
Soil type 13	1.65	1.35	2.03
			$p < .01$
Sheep not exposed to			
<u>Soil type 77</u>	1.57	1.15	1.74
Sheep exposed to			
Soil type 77	1.11	2.22	1.15
		$p < .01$	$p < .05$
Sheep not exposed to			
<u>Soil type 117</u>	1.51	1.25	1.61
Sheep exposed to			
Soil type 117	2.56	2.21	2.99
	$p < .05$		
<u>CLIMATE</u>			
	<u>inches</u>		
Average <u>annual rainfall</u>	< 40	1.83	1.48
on property of origin	41 - 60	1.36	1.33
	> 61	1.11	0.95
		$p < .01$	$p < .05$

Table XXIV Contd

		Prevalence (%) of sheep with small intestinal carcinomas		
		Total of all sheep inspected	Sheep whose whole life history was known	Sheep whose history was known for less than whole of life
<u>ALTITUDE</u>				
Mean <u>altitude</u> (feet above	< 200	1.64	-	1.69
sea level) of property	200-499	1.68	1.48	1.72
of origin	500-999	1.31	0.66	1.54
	> 1000	1.62	1.64	1.59
			p < .05	
<u>PASTURE SPECIES</u>				
Sheep not exposed to <u>dogstail</u>		1.34	0.98	1.47
Sheep exposed to dogstail		1.68	1.50	1.76
			p < .05	
<u>WEED SPECIES</u>				
Sheep not exposed to <u>dandelion</u>		1.77	1.40	1.90
Sheep exposed to dandelion		1.40	1.27	1.46
			p < .05	p < .05
Sheep not exposed to <u>fennel</u>		1.52		1.60
Sheep exposed to fennel		4.95		3.68
			p < .05	p < .01
Sheep not exposed to <u>stinging</u>				
<u>nettle</u>		1.71	1.42	1.81
Sheep exposed to stinging nettle		1.17	1.13	1.20
			p < .01	p < .01
Sheep not exposed to <u>californian</u>				
<u>thistle</u>		1.83	1.20	1.99
Sheep exposed to californian				
thistle		1.41	1.36	1.43
			p < .05	p < .01

Table XXIV Contd

Prevalence (%) of sheep with small intestinal carcinomas				
	Total of all sheep inspected	Sheep whose whole life history was known	Sheep whose history was known for less than whole of life	
Sheep not exposed to <u>water cress</u>	1.63	1.47	1.70	
Sheep exposed to water cress	0.85	0.71	0.98	
	p<.01			
Sheep not exposed to <u>Yorkshire</u> <u>Fog</u>	1.53		1.58	
Sheep exposed to Yorkshire fog	2.97	0.00	3.06	
	p<.05			
<u>STOCK MANAGEMENT</u>				
<u>Set versus mob stocking</u>	set	1.08	0.23	1.51
	equal	1.66	1.48	1.73
	mob	1.55	1.50	1.56
		p<.05		
<u>ANIMAL INSECTICIDES</u>				
Sheep not exposed to <u>carbophenothion</u>	1.47			1.54
Sheep exposed to carbophenothion	3.33	0.00		3.40
				p<.01
<u>LENGTH OF TIME SHEEP WERE ON</u> <u>PROPERTY FROM WHICH THEY</u> <u>WERE SLAUGHTERED</u>				
Whole of life	1.34			
4 years or more	1.33			
less than 4 years	1.79			
	p<.05			

T A B L E XXV

Comparison of the Number of Significant Chi Squared Results
in Table XXIII with Number of Significant Results Expected
Due to Chance

Total of all sheep

Number of chi squared tests performed = 119

				observed	expected by chance
Number of tests	p	<.05	>.01	7	4.8
" " "	p	<.01		3	1.2
" " "	p	>.05		109	113.0

$$\chi^2 = 3.72; \text{ d.f.} = 2; \text{ N.S.}$$

Sheep whose whole life history was known

Number of chi squared tests performed = 88

				observed	expected by chance
Number of tests	p	<.05	>.01	2	3.5
" " "	p	<.01		1	0.9
" " "	p	>.05		85	83.6

$$\chi^2 = 0.68; \text{ d.f.} = 2; \text{ N.S.}$$

Sheep whose history was known for less than whole of life

Number of chi squared tests performed = 112

				observed	expected by chance
Number of tests	p	<.05	>.01	3	4.5
" " "	p	<.01		5	1.1
" " "	p	>.05		104	106.4

$$\chi^2 = 10.73; \text{ d.f.} = 2; \text{ p} < .01$$

(b) Relationships Between Various Factors and Prevalence
of Small Intestinal Carcinomas

From the information obtained from vendors, all sheep were classified on the basis of their exposure, degree of exposure, or lack of exposure to various soil types, plant species, climatic factors, managerial regimes, agricultural chemicals and animal remedies. These data were also tabulated separately for sheep which had been bred by the vendor (i.e. their whole life history was known) and for sheep purchased by the vendor (i.e. their history was known for only part of the sheep's lives). These tabulations, together with chi squared values and probabilities of homogeneity of data, where calculated, are contained in Table XXIII. Chi squared values were not calculated where less than 500 sheep without intestinal carcinomas and less than seven with carcinomas were either exposed, or not exposed, to a particular factor. In calculating chi squared for some factors, data were pooled, as indicated by vertical bars in Table XXIII. Factors with which statistically significant variations in tumour prevalence ($p < .05$) were associated in these analyses are shown in Table XXIV, with tumour prevalence in sheep exposed, and not exposed, to the various factors expressed as percentages.

From the large number of chi squared tests carried out on data in Table XXIII, some "significant" ($p < .05$) results would be expected as chance occurrences. In Table XXV, numbers of "significant" results are compared with the numbers expected by chance, showing that in chi squared tests carried out on sheep whose history was known for less than whole of life, a greater number of tests revealed results of $p < .01$ than would be expected due to chance. In other analyses, the

T A B L E XXVI

Cross Tabulation of Data Pertaining to Average Annual Rainfall
and Presence or Absence of Dogstail on the Property from
which Ewes were Forwarded for Slaughter

(% of sheep with carcinomas of the small intestine)

		Presence or absence of dogstail on property of origin.	
		Absent	Present
Average annual rainfall (inches)			
on property of origin	< 40	2.035	1.713
	41 - 60	1.679	1.140
	61 - 80	1.238	0.968
	> 80	0.000	0.000

T A B L E XXVII

Tumour Prevalence in Slaughtered Ewes Forwarded by
Vendors in Groups of Varying Sizes

Number of ewes per line	Number of ewes inspected without tumours	Number inspected with tumours	Percent with tumours
< 30	593	12	1.98
30 - 99	4145	54	1.29
100 - 199	8038	111	1.36
200 - 299	4190	78	1.83
300 - 399	1650	27	1.61
400 - 499	2536	54	2.09
> 499	703	10	1.40

$$\chi^2 = 9.78; \text{ d.f. } = 6; \text{ N.S.}$$

T A B L E XXVIII

Prevalence of Tumours in Large and Small Lines of Ewes
where Lines of Both Over and Under Fifty were Inspected
from the same Vendor

Vendors code Number	Sheep in large lines		Sheep in small lines	
	Without tumours	With tumours	Without tumours	With tumours
27	250	5	34	1
47	121	1	17	0
59	120	2	41	0
82	170	3	16	2
139	105	0	11	0
147	144	3	5	0
202	278	6	35	0
204	87	1	40	1
Total	1,275	21	199	4
Prevalence	1.62%		1.97%	

number of significant chi squared results was not significantly different from the number expected due to chance.

A cross tabulation of data pertaining to annual rainfall and presence or absence of dogtail is shown in Table XXVI. Prevalence differences associated with these two factors appear, on the basis of this cross tabulation, to be independent of each other.

Relationship Between Line Size and Prevalence of Small Intestinal Carcinomas

Table XXVII compares the prevalence of small intestinal carcinomas in sheep forwarded for slaughter in lines of various sizes. Chi squared analysis showed no significant variation in tumour prevalence associated with variations in line size.

Prevalence of small intestinal carcinomas in large and small lines from the same vendors are compared in Table XXVIII. Few vendors had supplied sheep in lines of the two sizes (i.e. above and below fifty ewes) so that data available were insufficient for statistical analysis. The difference between tumour prevalences in sheep in the two categories of line size was small.

DISCUSSION

In disease investigations, it is usual to adopt as a means of diagnosis the most accurate techniques which are practicable in the circumstances. From the results of histological examination of lesions encountered during 1967, it was apparent that gross observation of lesions was an accurate means of diagnosis of carcinomas of the small

intestine in sheep. The further positive histological diagnosis in all forty-nine check cases examined histologically during 1968, together with consistently accurate diagnosis on gross examination of cases collected for more detailed pathological studies (Chapter II), confirmed that diagnosis on the basis of gross examination of viscera only, was sufficiently accurate to be used as the sole method in the collection of data for epidemiological studies of this neoplasm.

Examination of ewe viscera for small intestinal carcinomas revealed a prevalence of these neoplasms considerably higher than any previously recorded. The prevalence in the geographical areas in which these tumours were most common, was three times as great as the previous highest recorded prevalence in New Zealand (Webster, 1967), and was 5 - 6 times as great as the highest reported prevalence of small intestinal carcinomas in slaughtered ewes outside New Zealand (Norval, 1968) (page 11). The lowest prevalence found at any Meat Export Works in this investigation (0.2% at Marlborough) was slightly lower than the prevalence found at Edinburgh by Norval.

Webster (1967) considered that the minor modifications of the inspection techniques used in his previous survey (Webster, 1966) resulted in "very few, if any", neoplasms escaping detection, yet, in each of the following three years, three times as many small intestinal carcinomas were found in ewes slaughtered in the same area (Manawatu). This apparent increase in prevalence is attributed to an increase in sensitivity of diagnosis due to the personal examination of viscera for this one condition. This differed from the method described by Webster (1966) in which meat inspection staff, inspecting viscera, changed from time to time. They were responsible for the

examination of all visceral organs with the alimentary tract being viewed but not handled. For the detection of other than very large lesions, inspection of the intestines of sheep must involve their being palpated and manipulated to enable all portions of the intestine and its mesentery to be seen. Even with the improved inspection method used in these investigations it is probable that some very small carcinomas were not found. The increased number of small intestinal carcinomas found in the Manawatu district in 1968, compared to 1967 (Table XIII) is thought to have been due to an increased sensitivity of diagnosis accompanying increased practice with the inspection technique. The prevalence of neoplasms in ewes inspected in Manawatu during 1969 was intermediate between the 1967 and 1968 figures. Thus, there was no evidence of either an increasing or a decreasing tumour prevalence over these three years.

The impossibility of comparing data from this investigation with that of Webster illustrates the need in investigations such as this, for the person collecting the data to be keenly aware of the need for his vigilance and, if possible, for one interested person to perform all inspections. This point was further emphasised when an attempt was made to extend the detailed epidemiological investigation to include sheep slaughtered in South Canterbury by utilising a technician specially trained in the techniques of examination of abdominal viscera. A change in personnel part way through the collection of data in that region was accompanied by an immediate decrease in diagnosis of intestinal neoplasms in British breed sheep from approximately 1% to 0.5%, and, consequently, that part of the investigation was abandoned.

T A B L E XXIX

The Prevalence of Carcinomas of the Small Intestine
in Ewes Forwarded for Slaughter in Lines of
Various Sizes (Webster's data)

Number of ewes per line	Number of ewes inspected without carcinomas	Number inspected with carcinomas	Percent with carcinomas
30	6184	48	0.776
30 - 99	15,590	60	0.385
99	35,753	141	0.394

$$\chi^2 = 18.2 ; \quad df = 2; \quad p < .001$$

The validity of an epidemiological investigation such as this also depends on the tumour prevalence in the sheep inspected from different farms being a relatively accurate representation of the prevalence in all sheep of comparable age on that farm. Webster (1967) considered that the very high prevalence of neoplasms (including small intestinal carcinomas) in lines of less than thirty ewes, shown in his Tables 3 and 4, resulted from these sheep being, almost exclusively, animals culled for reasons of poor condition and/or health. It was suggested that farmers were able to detect signs of ill health in sheep with small intestinal carcinomas and that these were forwarded for slaughter with other unhealthy sheep, in separate groups from the healthy "cast for age" ewes. Re-examination of Webster's data revealed that only 40,478 of the 62,235 ewes examined in his survey were represented in his Tables 3 and 4, while virtually all neoplasms were included. Checking of original data, made available by Webster, showed that he had included in Tables 3 and 4 only those lines of ewes in which neoplasms had been diagnosed. Thus a considerable bias was introduced favouring a high incidence in small lines. Retabulation of part of his data pertaining to intestinal carcinomas is shown in Table XXIX. This Table contains only those lines of ewes forwarded for slaughter in the name of a farmer vendor. It does not include lines of ewes killed under the name of a Meat Export Works or non-farming companies, as such lines may have been pooled subsequent to purchase, and therefore might not reflect the true size of the groups forwarded from individual farms. Chi squared analysis of Webster's corrected data shows there was a tendency toward higher tumour prevalence in lines of less than 30 ($p < .001$),

but this difference was much less than appeared from Webster's published tables.

In the present study, analyses failed to show any significant variation in tumour prevalence between lines of different sizes (Table XXVII). A comparison of tumour prevalence in lines of different sizes submitted by the same vendors would have been a better test of Webster's hypothesis. These data, which were available from only eight farms in the present investigation, have been collected in Table XXVIII. However, the numbers of small intestinal carcinomas recorded, and expected, in the small lines were too small for statistical analysis. Any bias in tumour prevalence in small lines resulting from selection of diseased sheep, as suggested by Webster, must have been so small in this investigation as to have been of no consequence. Therefore, it was considered legitimate to use all data collected in the overall analyses.

Geographic Distribution of Carcinomas of the Small Intestine in Sheep

The largest variation in prevalence of carcinomas of the small intestine was between British breed sheep (predominantly Romneys) and "fine wool" ewes (Merino, Merino cross and Corriedale breeds) (Tables XIV and XV). Conditions under which these breed types are usually farmed in New Zealand differ greatly, and it is not possible to state whether the difference noted was due to a difference in susceptibility of the breeds to a carcinogen, or to a difference in degree of exposure to one or more carcinogens. The presence of between districts variations in prevalence of small intestinal carcinomas amongst British

breed ewes (Tables XVI and XVII) suggested that there was probably a variation in the contact sheep had with the agent(s) responsible for these neoplasms. Although it was shown in Table XX that significant differences in tumour prevalence existed in ewes from different farms, it is considered unlikely that between districts variations resulted from the examination of ewes from unrepresentative high or low tumour prevalence farms. The average number of ewes examined per consignment during 1969 was 109 in Manawatu and 121 in Wairarapa. If consignments were of similar size in other districts, sheep inspected would have come from a sufficient number of farms to be reasonably representative of the district. The difference in prevalence of small intestinal carcinomas between sheep inspected in the Wairarapa and those inspected slaughtered in the Manawatu region (Table XVII) reveals itself again in Figure 71, in which the majority of farms east of the central North Island ranges had a low tumour prevalence, whereas most of those west of that range had a high prevalence of carcinomas. It is considered that there is sufficient evidence to justify the conclusion that environmental and/or genetic differences were causing a difference in the incidence of carcinomas of the small intestine in sheep in different parts of New Zealand.

The Effect of Environment on the Prevalence of
Small Intestinal Carcinomas

In planning an epidemiological investigation such as that instituted during 1969, a decision must be made whether to cover large numbers of animals and farms, and accept inaccuracies in the traceback histories of these animals, or to obtain prevalence data

on a smaller number of animals and farms, thus enabling a closer examination of their histories and recent environment. In this study it was possible to obtain histories regarding many factors only by questioning sheep vendors. Histories could be obtained only for the periods that vendors had owned the ewes, previous owners generally being unknown. While the status of farms in some respects (e.g. annual rainfall, mean altitude, farm size, average stocking rates) could be obtained with relative accuracy, there would be considerable errors in answers to some other questions (e.g. weeds species present, less well known pasture species, and disease histories). These limitations led to the decision to base this investigation on the maximum number of ewes practicable, and, while taking reasonable precautions to minimise sources of error, to accept that such inaccuracies must occur. Errors in replies would generally have tended to minimise, rather than exaggerate, real prevalence differences between sheep with different backgrounds. Few false positive replies are likely to have been given to questions on pasture and weed species, for example, but false negative replies might have been numerous, especially regarding inconspicuous weed species. Thus, if a high tumour prevalence was associated with the presence of a particular factor, the prevalence in sheep from vendors acknowledging the presence of that factor would be representative, but the prevalence of tumours in sheep from farmers not recognising the presence of the factor would be artificially high.

The diagnosis of at least one carcinoma of the small intestine in all 32 lines from which over 185 ewes were inspected indicates that the factors responsible for inducing these neoplasms must be widespread in the New Zealand sheep farming environment. Tabulation and analysis

of data in Table XX, however, revealed significant heterogeneity of tumour prevalence in sheep originating from different farms, and further analyses in Tables XXI, XXIII, and XXIV showed statistically significant ($p < .05$) variations in tumour prevalence to be associated with a total of sixteen factors.

For variations to be thought of possible aetiological significance it was considered that the following factors should hold : -

- (1) Exposure to a factor should have been associated with a significantly higher tumour prevalence, not a lower one.
- (2) The significant variation in prevalence of carcinomas of the small intestine should be reflected in the variation in prevalence in sheep whose whole life histories were known. Prevalence differences amongst sheep subjected to the influence of a factor for the whole of their lives, or known to have not been exposed to a factor at any time, should represent the potential effect of that factor more accurately than differences amongst sheep whose histories were known for only part of their lives.
- (3) With data separated on the basis of graduated exposures to a factor (e.g. altitude, rainfall, temperature), the tumour prevalence should either increase or decrease with gradation of the factor but not show a peak or trough.

- (4) More than 20% of tumours should have been found amongst ewes exposed to the factor with which the significant variation in prevalence was associated. It is unlikely that factors with which only small numbers of tumours were associated could have contributed to the aetiology of a significant proportion of all neoplasms encountered in the investigation.

On these bases, only crested dogstail, average annual rainfall, use of potassic fertilisers, and variations in length of time sheep had been on the property from which they were slaughtered could be considered likely to be related to the aetiology of this disease.

Comparisons of the actual number of significant ($p < .05$) chi squared results in Tables XXIII and XXIV with the number expected by chance (Table XXV) indicated that the differences associated with crested dogstail, annual rainfall and variations in length of time sheep had been on the property from which they were slaughtered could have been due to chance. However, it is not impossible that one or more of these differences is a meaningful one, and for that reason the possible aetiological significance of the above differences will be discussed.

The Aetiological Significance of Factors Associated
with Variations in Prevalence of Carcinomas of the
Small Intestine

(a) Crested dogstail

Amongst sheep slaughtered from farms on which they had been bred, there was a 3 : 2 ratio in the prevalence of carcinomas of the

small intestine between sheep supposedly exposed and not exposed to crested dogtail (Cynosurus cristatus). Only one "high prevalence" farm did not have crested dogtail reported in its pastures, whereas dogtail was reported to be present in only one half of low prevalence farms (Table XXI, Figure 71). The morphology of crested dogtail plants is not dissimilar to that of ryegrass species and it is probable that a considerable number of farmers were unaware of the presence of this species in their pastures. The seed heads of crested dogtail, on the other hand, are characteristic and readily recognisable. The apparent association of carcinomas with crested dogtail might reflect an association with seed heads or an association with grazing practices which allowed seed heads of crested dogtail to develop. Crested dogtail is probably less common in areas where "fine wool" sheep are commonly farmed, but no such difference is thought to exist between areas from which British breed ewes were inspected.

While it is possible that either crested dogtail foliage or seed heads contain carcinogens it is also possible to speculate that the apparent association of intestinal carcinomas with dogtail is an indirect one resulting from management procedures allowing the recognition of dogtail seed heads in the pasture. These management practices might affect tumour prevalence directly, or they might allow the development of pasture and soil conditions favourable for the development of other carcinogenic factors (e.g. other plant species or fungi).

(b) Potassic fertilisers

The significantly greater proportion of high prevalence farms

to which potassic fertilisers had been applied was consistent with the greater use of potassic fertilisers in the North Island, in which there was also a higher prevalence of small intestinal carcinomas. The area of land in pasture is twice as great in the North Island as in the South (Table 36, New Zealand Farm Production for the Season 1967-68) and this is reflected in the quantities of phosphatic fertilisers used (Table 5, Statistical Review of Fertilisers in New Zealand 1968 - 69). However, approximately nine times as much potassic fertiliser is used in the North Island as in the South Island, indicating an average application rate in the North Island of approximately 4.5 times that in the South Island (Table 7, Statistical Review of Fertilisers in New Zealand 1968 - 69).

Over 90% of New Zealand's potassic fertilisers are imported as muriate of potash, a processed form of mined potassium chloride still containing considerable quantities of impurities. While it is unlikely that KCl is carcinogenic, impurities in the commercial product may be. It is also possible that applications of potash could stimulate the growth of plant species which have carcinogenic properties.

If further studies of the epidemiology of carcinomas of the small intestine of sheep confirm this association with the use of potassic fertilisers, experiments involving the oral and parenteral administration of muriate of potash would seem warranted.

(c) Average annual rainfall

The higher prevalence of small intestinal carcinomas amongst sheep from farms with lower rainfall (Table XXIII) was not supported by any rainfall differences between the selected high and low

prevalence farms (Table XXI). The trend in the "between district" data (Tables XIII and XVI) would have been for higher prevalence in areas of higher rainfall. The contradictory nature of these results suggest that the apparent rainfall effect in Table XXIII was probably due to chance and was not related to the epidemiology of the disease.

(d) Sheep stocking density

In Tables XVIII and XIX there is a strong positive correlation between prevalence of carcinomas of the small intestine in geographic areas and average sheep stocking rates. Differences in stocking rates are, however, usually associated with differences in soil types and fertility, pasture and weed species, managerial factors and the use of agricultural chemicals and animal remedies. An increased prevalence of neoplasms associated with increased grazing density of sheep, independent of correlations with other factors, would be consistent with an hypothesis that the cause of the neoplasms was horizontally transmitted in nature.

Ewes known to have spent their whole lives on farms with average sheep stocking rates of less than three per acre had a lower prevalence of carcinomas (0.92%) than sheep farmed at higher densities (1.41%), but, as only 1088 sheep were in the former category, this difference was not statistically significant.

In the 836 sheep inspected which were known to have been predominantly "set stocked" throughout their entire lives, only two (0.24%) carcinomas of the small intestine were diagnosed ($p < .05$). Amongst sheep whose histories were known for only part of their lives there was no difference in tumour prevalence associated with "set"

and "mob" stocking practices (Table XXIII). This lack of variation might be due to "set stocked" sheep having been "mob" stocked earlier in their lives and the effects of this on prevalence of carcinomas continuing up till the time of slaughter.

There are sufficient indications in these observations to suggest that increased sheep grazing density could possibly have affected prevalence of small intestinal carcinomas, but the evidence is insufficient to justify a conclusion that sheep proximity per se was directly associated with an increased prevalence. This matter warrants further investigation.

- (e) Length of time sheep were on the property from which they were sent for slaughter

Variations in the length of time sheep had been on the vendors' property reflected differences in management practices. Ewes which had been bred by the vendor came from farms on which sheep of all ages were present, ewe lambs being retained to replace older ewes that had died or were sold. Vendors owning sheep for more than four years would usually purchase females from 6 - 18 months of age and retain these for breeding. Ewes owned by vendors for less than four years had generally been purchased by the vendor at approximately five years of age and retained by him for either one or two breeding seasons. Lambs from this latter type of farm would commonly be sold as "fat lambs" when 4 - 6 months of age, almost the only sheep on these farms for the remainder of the year being 5 - 6 year old ewes. From Table XXIII, it can be seen that ewes from this latter type of farm had a higher prevalence of carcinomas of the small intestine than other ewes.

Over most of the North Island, the majority of slaughtered ewes inspected during 1967 and 1968 would have come from "fat lamb" farms, as was the case in Manawatu during 1969. A more common managerial practice in the South Island is for ewes to be purchased by "fat lamb" farmers at 12 - 18 months of age, many from "high country" farms with improved tussock lands. Thus, the histories of British breed sheep inspected in the South Island may have resembled to a greater extent the histories of those sheep inspected during 1969 which had spent at least four years on the vendors' properties. The environment in which a proportion of South Island British breed sheep spend the early part of their lives would also be more similar, in some respects (climate, soil types, and some pasture and weed species), to the environment in which "fine wool" ewes are farmed. From the data available, it appears possible that some factor associated with "fat lamb" farming practices, which involve the purchase of ewes at about five years of age, using them for lamb production for one or two seasons, and then slaughtering, may result in an elevation of tumour incidence.

Factors Suggested of Aetiological Significance
by Previous Authors

(a) Phenothiazine

Webster (1966) suggested that fine particle phenothiazine (thiodiphenylamine) might be the cause of neoplasms in sheep, including carcinomas of the small intestine. No experimental evidence is available to support this suggestion, nor is there evidence that diphenylamine, from which phenothiazine may be manufactured (British Veterinary Codex, 1965) is carcinogenic. However, the carcinogen 4-

aminodiphenyl is a contaminant of commercial diphenylamine (Clayson, 1962), and hence there is a possibility that phenothiazine, as prepared, could contain a small proportion of this chemical.

Phenothiazine usage has decreased markedly since 1962-63 and only 394 of the ewes inspected during 1969 were recorded as having been drenched with this product. If phenothiazine had been responsible for an appreciable proportion of sheep neoplasms, the prevalence should have decreased over the last 4 - 5 years. There is no evidence to suggest that such a decrease has occurred.

(b) Bracken fern (Pteridium aquilinum)

Following the demonstration that Pteridium aquilinum was a potent carcinogen, causing neoplasms of the intestine of rats (Evans and Mason, 1965) (Chapter V), it was suggested by McCrae (1968) that ingestion of this plant species might have been responsible for the carcinomas of the intestines of sheep observed by him on the North York Moors. Evans (1968) lent support to this suggestion by reporting that one of two sheep fed bracken fern had developed a carcinoma of the large intestine.

From results of investigations recorded in this chapter (Table XXI and XXIII) it appears unlikely that Pteridium aquilinum var. esculentum played any significant part in the causation of neoplasms encountered in this study. Although the prevalence of carcinomas of the small intestine was slightly higher in ewes from properties on which bracken fern was common than in sheep from properties where it did not grow (2.08% versus 1.54%), and similar prevalence differences were present in sheep whose whole life histories were known (Table XXIII), eight of the 14 high tumour prevalence farms were recorded as having no bracken fern (Table XXI).

This epidemiological study of small intestinal carcinomas in sheep has not shown any particular factor or factors which can be said to be directly or indirectly involved in the aetiology of these neoplasms. A number of factors, particularly breed susceptibility, crested dogstail, potassic fertilisers, intensity of sheep farming and the practice of transferring five year old ewes to "fat lamb" farms have, however, been shown to be statistically associated with higher tumour prevalences, and these deserve further investigation. The negative results revealed in analyses of data concerning agricultural chemicals and animal remedies are important in as much as they, in part at least, absolve these agents from possible incrimination as carcinogens in the rural environment.

The most effective means of checking the repeatability of the prevalence variations found in this investigation would be inspection of greater numbers of large lines of ewes (e.g. exceeding 200), the selection from these of 'high' and 'low' prevalence groups and the tracing of the detailed histories of these groups with regard to the factors of interest.

Further long term investigations of the epidemiology of this condition might best be directed toward a prospective epidemiological approach, in which fewer farms are selected, and from which greater numbers of sheep could be inspected. Post mortem examination of ewes dying on these properties, together with the examination of 2,000 slaughtered ewes from each, over a period of approximately five years, should provide an accurate measure of the incidence of small intestinal carcinomas. Restricting such an investigation to 20 - 30 farms would enable accurate information to be obtained on physiographic, botanical

and managerial factors on these farms and analysis of data obtained should reveal any tumour prevalence differences which exist. In view of the potential importance of these neoplasms to New Zealand's international meat trade, the country's sheep farmers and our public health, such exhaustive investigations are fully warranted.

SUMMARY

Viscera of ewes slaughtered at ten Meat Export Works in seven geographic areas of New Zealand during 1967 and 1968 were examined for carcinomas of the small intestine. Analyses showed that British breed ewes had a significantly higher prevalence of small intestinal carcinomas than did Merino, Merino cross or Corriedale ewes. There were also significant variations in tumour prevalence between British breed sheep killed in different areas. The prevalence of these neoplasms in slaughtered British breed sheep varied from approximately 1.5% to 0.9%, while the prevalence in fine wool breeds was 0.5% and 0.2% in the two groups of such sheep examined. Whether these differences were due to environmental or genetic factors could not be determined.

Analyses of the histories of 22,201 ewes with 346 carcinomas of the small intestine from 191 vendors, inspected during 1969, revealed significant variations ($p < .05$) in prevalence of carcinomas associated with 16 of the 153 factors examined. On the basis of a number of defined criteria, all but four of these factors were considered unrelated to the aetiology of the neoplasms. The remaining factors were crested dogstail, potassic fertilisers, rainfall and the length of time sheep

had been on the property from which they were forwarded for slaughter. The possible aetiological role of each of these factors was discussed but no firm conclusions regarding the aetiology of carcinomas of the small intestine were reached. The possible relationship between prevalence of carcinomas of the small intestine in different areas and the average sheep stocking rates in these areas was discussed, together with the findings regarding stocking rates on individual farms from which ewes were examined. Again no firm conclusions could be drawn regarding any effect stocking rates might have had on prevalence of neoplasms. Because phenothiazine (thiodiphenylamine) and bracken fern (Pteridium aquilinum var. esculentum) had previously been suggested as possible causes of small intestinal carcinomas in sheep, these two factors were investigated. Findings indicated that neither of these agents was likely to have been responsible for a significant proportion of the carcinomas diagnosed in this investigation.

C H A P T E R I V

CARCINOGENICITY OF CERTAIN CHEMICALS ADMINISTERED TO SHEEP

INTRODUCTION

Many chemicals are capable of inducing neoplasia in animals but relatively little is known concerning the relative susceptibility of sheep to these agents. On the basis of any apparent relative scarcity of ovine neoplasms, Feldman (1932) and Pamukcu (1956) suggested that sheep might be relatively insusceptible to neoplasia. In this section, this hypothesis is tested by experimental administration of certain selected carcinogens to sheep.

Chemical Carcinogens Previously Administered to Sheep

Experiments by de Kock (1958), in which coal tar, methylcholanthrene, dibenzanthracene and benzpyrene were administered to groups of sheep either weekly, on alternative weeks or monthly, resulted in the development of only one neoplasm. This occurred in an animal which had been treated with benzpyrene by both intravenous and intrapulmonary routes and was diagnosed as either an endothelioma or mesothelioma of the pleura. Lloyd (1966) injected 9,10-dimethyl -1, 2-benzanthracene into subcutaneous dermal cysts of sheep and also supplied the chemical to the skin of normal and cyst-bearing sheep. This treatment resulted in the development of squamous cell carcinomas in 40% of cysts. Papillomas developed on the skin of both, cyst-bearing and normal sheep, but more

frequently and earlier on the former (page 7).

Nasal tumours developed in two of five sheep fed ground nut meal containing aflatoxin (Lewis et al., 1967) (page 5). The feeding of radioiodine to 19 sheep resulted in the development of thyroid adenomas in 15 of these and a fibrosarcoma in one other (Marks and Bustad, 1963).

The hepatotoxic effects of dimethylnitrosamine in sheep were demonstrated by Sakshaug, Sognen, Hansen and Koppang (1965) who found a single oral dose, calculated at 5 mg/kg, killed sheep in 11 days. No long term observations following sublethal dosage were reported, so the susceptibility of sheep to the carcinogenicity of this compound is unknown.

Carcinogens used in this experiment

Chemicals chosen for use in this experiment were 20-methylcholanthrene, 3:4 benzpyrene, urethane (ethyl carbamate) and N-methyl-N-nitro-N-nitrosoguanidine (nitrosoguanidine).

Methylcholanthrene : A wide range of tumours has been produced in laboratory animals by methylcholanthrene, including tumours of the skin (Berenblum and Haran-Ghera, 1957 b), blood vessel endothelium (White and Stewart, 1942), mammary gland (Bonser, 1954), liver (Bain, Allen, Silbermann and Kowalewski, 1959; Pietra, Rappaport and Shubik, 1961), lung (Lorenz and Stewart, 1940; Pietra et al., 1961), lymphoid tissue (Pietra et al., 1961), stomach (Stewart and Lorenz, 1941b, 1942; Howes and de Olivera, 1948; Zaldivar, 1964) and caecum (Rack, Kaufman and Simeone, 1955). Small intestinal adenocarcinomas have been produced in mice by

administration of methylcholanthrene either in drinking water or mixed with food (Stewart and Lorenz, 1941 a; White and Stewart, 1942; Lorenz and Stewart, 1940, 1947).

Benzpyrene : Benzpyrene has also been reported as producing a wide range of tumours in laboratory animals, including neoplasms of the lungs, mammary gland, liver (Pietra et al., 1961), brain and smooth muscle (Zimmerman and Arnold, 1943). Many of the studies using benzpyrene have concerned the skin (Glucksman, 1945; Berenblum and Haran-Ghera, 1957 b), though it was used by Stewart and Lorenz (1942) to produce gastric tumours in mice.

Urethane : Urethane was at first thought to have a specific action in producing pulmonary adenomas in laboratory animals (Nettleship and Henshaw, 1943; Jaffé, 1944, 1947), but is now recognised as capable of inducing many types of neoplastic lesions (Tannenbaum and Silverstone, 1958; Pietra et al., 1961; Tannenbaum, Vesselinovitch, Maltoni and Mitchell, 1962). It was suggested by Tannenbaum and Maltoni (1962) that urethane acts by enhancing or potentiating carcinogenesis, thus increasing the incidence and decreasing the age of occurrence of neoplasms which occur "spontaneously" in the particular species or strain of animal. This is in contrast to the other three carcinogens used in this experiment in that they are considered to be fully competent carcinogens capable of initiating and promoting the growth of neoplasms not normally seen in the species under observation.

N-methyl-N-nitrosoguanidine : The range of neoplasms produced by the N-alkyl-N-nitroso group of compounds includes tumours of the liver (Magee and Barnes, 1956), lung (Schoental and Magee, 1962; Druckrey and Preussman, 1962), stomach (Druckrey, Preussman, Schmahl and Muller, 1961; Schoental and Magee, 1962), oesophagus (Schoental and Magee, 1962; Druckrey, Preussman, Blum, Ivankovic and Afkham, 1963), subcutaneous connective tissues (Herrold, 1966), and intestinal tract (Herrold, 1969). Carcinomas of the small intestine were produced by the intraperitoneal administration of N-ethyl-N-nitrosourethane to rats (Schoental, 1965), and by intravenous, intraperitoneal and intragastric administration of N-methyl-N-nitrosourea. The related compound N-methyl-N-nitro-N-nitrosoguanidine (nitrosoguanidine), a very potent mutagen, has produced fibrosarcomas and rhabdomyosarcomas after subcutaneous injections (Sugimura, Nagao and Okado, 1966), and only three to five oral doses of this chemical were required to induce carcinomas of the small intestine in rats (Schoental, 1966). Sugimura, Kogure, Oboshi and Sugimura (1970) produced a range of neoplasms in the stomach, intestines and livers of rats by including nitrosoguanidine in their drinking water. Carcinomas of the duodenum, jejunum and colon were amongst the neoplasms diagnosed. This carcinogenic action of nitrosoguanidine is in agreement with the observation by Magee and Lee (1964) that mutagenic potency and carcinogenicity are positively correlated within this group of compounds.

Selection of the above four chemical carcinogens for use in these experiments was both on the basis of their known ability to produce a wide range of neoplasms and on the likelihood of their producing neoplasms in the gastrointestinal tract. Methylcholanthrene,

benzpyrene and nitrosoguanidine have all produced carcinomas of the small intestine in laboratory animals, and, if the suggestion by Tannenbaum and Maltoni (1962) that urethane enhances the development of tumours to which the species is "naturally" susceptible is true, then in sheep this chemical might be expected to lead to the development of considerable numbers of small intestinal carcinomas.

MATERIALS AND METHODS

In the order in which they were born, 101 Romney lambs were randomised into six groups of each sex (i.e. twelve groups). One group of each sex received each of the following treatments : -

- (a) Nil (controls)
- (b) Intraperitoneal injections of 20-methylcholanthrene as a 10 mg/ml solution in peanut (arachis) oil at the rate of 5 mg/Kg each week.
- (c) Intraperitoneal injections of 3 : 4-benzpyrene as a 10mg/ml solution in peanut oil at the rate of 5 mg/Kg each week.
- (d) Intraperitoneal injections of urethane as a 50 mg/ml solution in water at the rate of 25 mg/Kg each week.
- (e) Intraperitoneal injections of nitrosoguanidine as a 10mg/ml solution in water at the rate of 5 mg/Kg each week.
- (f) An injection of 5 mg of methylcholanthrene, in a 50 mg/ml solution/suspension in peanut oil, into the wall of the small intestine. This treatment was administered via a ventral laparotomy, with the aid of local anaesthesia, when the lambs were 12 - 17 weeks old.

T A B L E XXX

Age of Control Sheep at Time of Death

	Identification	Age at Death (weeks)	Lesions associated with treatment
MALES	178/179	218	*
	234/235	191	*
	160/161	190	*
	213/214	187	*
	219/220	222	*
	298/299	7	**
	266/267	189	*
	1783/1784	41	**
FEMALES	180/181	180	*
	226/227	216	*
	242/8836	220	*
	170/171	180	*
	8839/212	222	*
	268/269	179	*
	143/144	179	*
	278/279	189	*
	8840/1793	190	*

* Sheep killed and necropsied at termination of experiment.
No neoplasms were found.

** Death due to a variety of diseases considered unrelated
to the experiment.

T A B L E XXXI

The Effect of Treating Sheep with Intraperitoneal
Injections of Methylcholanthrene

	Identification	Number of Weekly Treatments	Total Dose (mg)	Age at Death (weeks)	Lesions associated with treatment
MALES	197/198	12	932	215	*
	236/237	12	1007	180	*
	162/163	11	667	180	*
	205/206	7	402	7	**
	0248/255	11	587	190	*
	276/277	9	417	9	Hepatotoxicity ***
	294/295	10	557	222	*
	1786/1787	9	530	197	*
FEMALES	119/120	11	672	11	Hepatotoxicity
	195/196	12	632	215	*
	248/249	12	642	220	*
	172/173	11	635	188	*
	252/253	11	547	190	*
	133/134	3	72	3	**
	127/128	10	377	178	*
	131/132	9	425	9	Hepatotoxicity
	1776/1777	6	185	6	Hepatotoxicity

* Sheep killed and necropsied at termination of experiment.
No neoplasms were found.

** Death due to a variety of diseases considered unrelated
to the experiment.

*** Toxic liver injury - see page 93.

T A B L E XXXII

The Effect of Treating Sheep with Intraperitoneal
Injections of Benzpyrene

	Identification	Number of Weekly Treatments	Total Dose (mg)	Age at Death (weeks)	Lesions associated with treatment
MALES	115/116	15	1090	218	*
	193/194	15	1067	191	*
	240/241	15	905	112	Peritonitis
	164/165	14	1095	188	*
	217/218	14	1117	129	Mesothelioma***
	225/251	14	967	222	*
	262/251	14	902	181	*
	137/138	12	562	80	**
	1797/1798	10	610	212	*

FEMALES	228/229	15	842	86	Peritonitis
	232/233	15	837	137	Mesothelioma
	166/167	14	690	81	**
	258/259	14	700	217	*
	264/265	14	887	50	Mesothelioma
	145/146	13	820	189	*
	292/293	13	732	188	*
	1723/1724	11	440	216	*

* Sheep killed and necropsied at termination of experiment.
No neoplasms were found.

** Death due to a variety of factors considered unrelated to
the experiment.

*** See pages 91 - 93.

T A B L E XXXIII

The Effect of Treating Sheep with Intraperitoneal
Injections of Urethane

	Identification	Number of Weekly Treatments	Total Dose (mg)	Age at Death (weeks)	Lesions associated with treatment
MALES	182/183	15	5487	222	*
	238/239	15	5000	191	*
	207/208	14	4325	179	*
	221/222	14	4725	181	*
	280/281	13	4200	189	*
	282/283	13	4337	189	*
	1792/0070	12	4325	194	*

FEMALES	117/118	15	5337	181	*
	187/188	15	1787	215	*
	152/153	15	5187	191	*
	156/157	14	5237	188	*
	153/169	14	3700	220	*
	209/210	14	4937	180	*
	141/142	13	4387	188	*
	296/297	13	3562	180	*
	272/273	13	3262	178	*

* Sheep killed and necropsied at termination of experiment.
No neoplasms were found.

T A B L E XXXIV

The Effect of Treating Sheep with Intraperitoneal
Injections of Nitrosoguanidine

	Identification	Number of Weekly Treatments	Total Dose (mg)	Age at Death (weeks)	Lesions associated with treatments
MALES	121/122	11	877	181	*
	230/231	8	440	217	*
	244/245	8	360	22	Peritonitis ***
	203/204	7	312	8	Peritonitis
	223/224	7	375	221	*
	129/130	6	290	183	*
	135/136	3	82	3	*
	1795/1796	4	107	6	Peritonitis

FEMALES	176/177	8	300	13	Peritonitis
	189/190	8	302	218	*
	246/247	8	367	19	Peritonitis
	201/202	7	470	190	*
	256/257	7	387	82	**
	285/287	6	237	120	Peritonitis
	126/300	6	285	222	*
	274/275	1	25	1	**
	1719/1720	1	25	216	*

* Sheep killed and necropsied at termination of experiment.
No neoplasms were found.

** Deaths due to a variety of diseases considered unrelated
to the experiments.

*** See page 93.

T A B L E XXXVThe Effect of Treating Sheep with Intramural Injections of
Methylcholanthrene into the Intestinal Wall

	Identification	Age When Treated (weeks)	Age at Death (weeks)	Lesions associated with treatment
MALES	184/185	17	216	*
	199/200	16	192	*
	1386/155	17	189	*
	174/175	16	190	*
	260/261	15	190	*
	139/140	15	189	*
	288/289	14	212	*
	1382/1785	13	187	*

FEMALES	113/114	17	187	*
	191/192	17	192	*
	250/251	16	189	*
	158/159	15	220	*
	215/216	16	191	*
	290/291	14	15	Ventral hernia through surgical incision
	270/271	15	189	*
	284/285	14	190	*
	1721/1723	12	221	*

* Sheep killed and necropsied at termination of experiment.
No neoplasms were found.

Treatments (b) to (e) were commenced when lambs were 2 - 9 days old. It had been intended that they continue for 25 weeks. However, deaths attributable to treatments forced the cessation of further treatments at the times shown in Tables XXXI to XXXIV.

Tables XXX to XXXV show the number of sheep of each sex in each treatment group, the number of weekly treatments, the total dose of carcinogen and the age at death of each lamb.

Male lambs were castrated when 3 - 7 weeks old. All lambs were vaccinated twice with a multiple clostridial vaccine* and were dosed orally eight times between six months and $2\frac{1}{2}$ years of age with an anthelmintic**. Lambs were weaned at approximately 16 weeks of age and were grazed on a predominantly ryegrass/white clover pasture.

All animals dying during the course of the experiment, or slaughtered at its termination were subjected to necropsy and all lesions suggestive of neoplasia were examined histologically.

RESULTS

Details concerning numbers of treatments, total dose of carcinogen administered, age at death and lesions found which were possibly associated with the treatments are shown in Tables XXVI to XXXV.

The only neoplasms found were in three animals to which benzpyrene had been administered (Table XXXII). These sheep presented with their abdomens distended by ascitic fluid. At necropsy, it was

* Ovilin. Glaxo Laboratories (N.Z.) Ltd.

** Thibenzole. Merck, Sharp and Dohme (N.Z.) Ltd

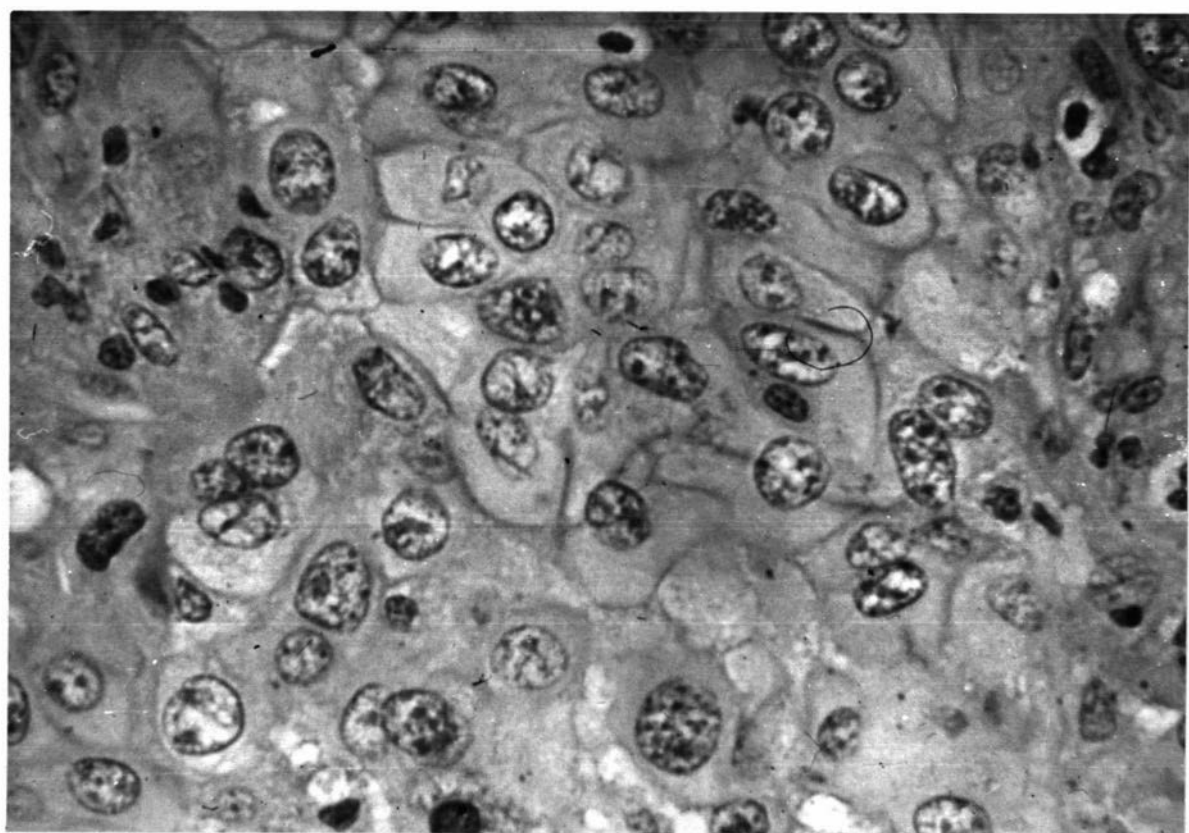
following page 91

Figure 72

A portion of the intestines and omentum of a sheep with an experimentally induced mesothelioma. The peritoneal surfaces are covered by small nodules.

Figure 73

Section of a nodule of an experimentally induced mesothelioma showing cells with large, clear nuclei, abundant cytoplasm and clearly defined cell boundaries. H & E. x 650



following page 91

Figure 74

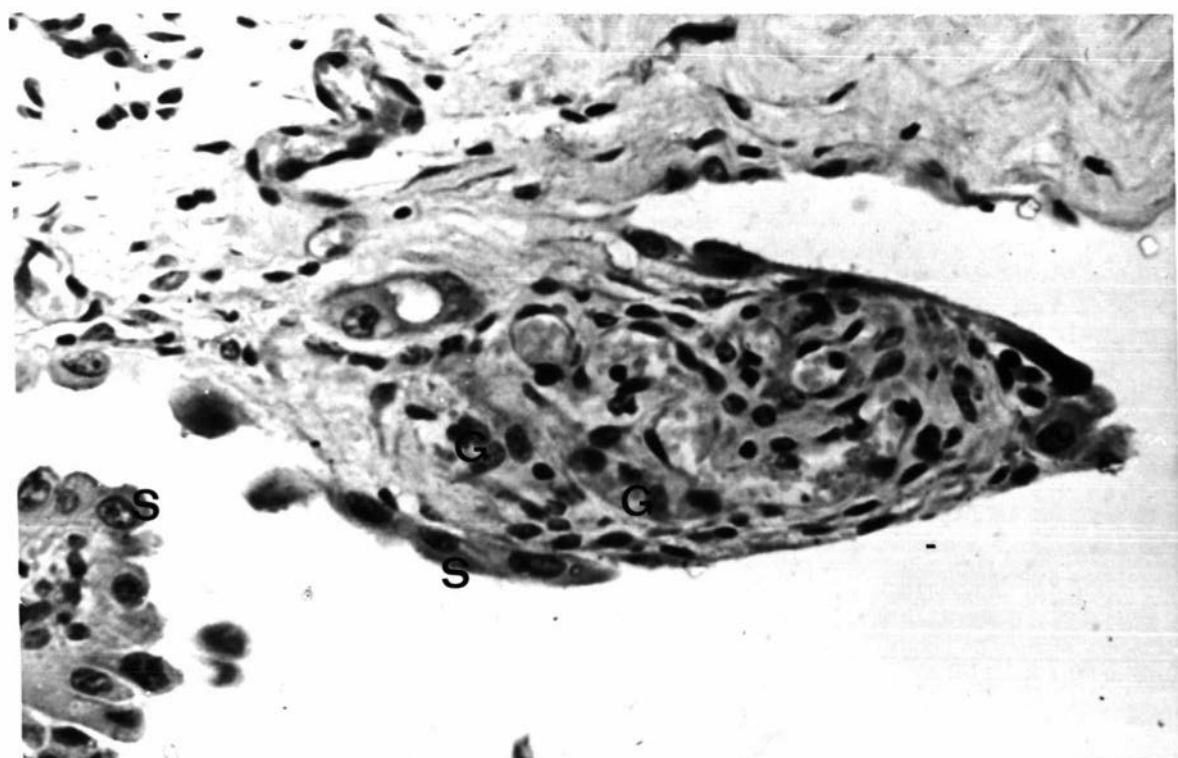
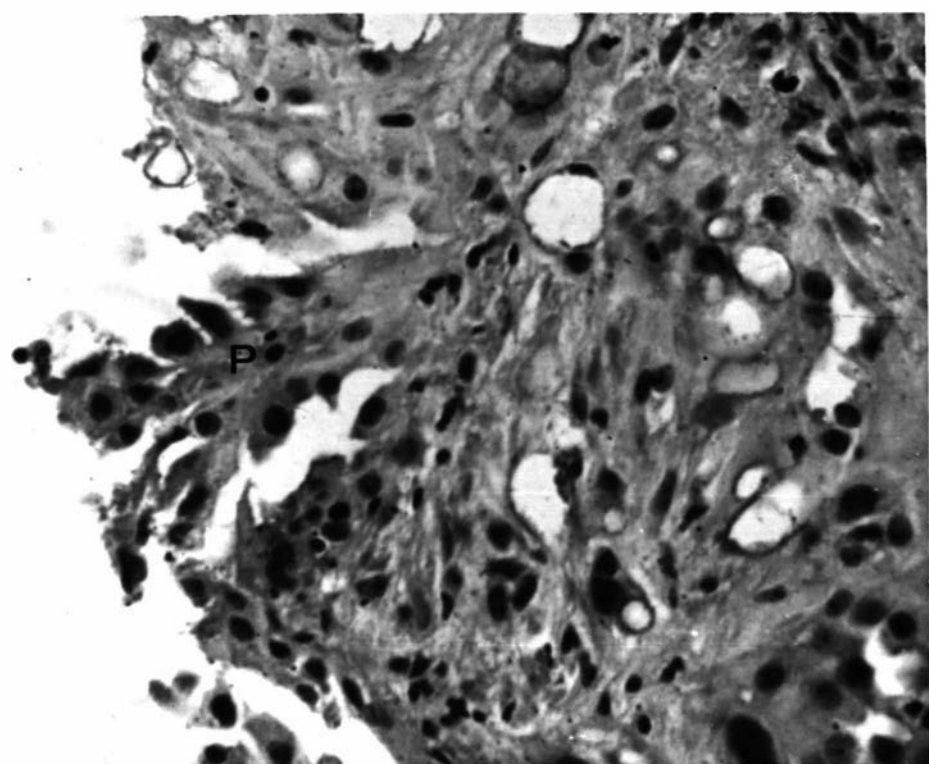
Surface of mesothelioma showing a small papilliform structure

(P). H & E. x 350

Figure 75

Mesothelioma showing a single layer of cells on the surface
(S) and small groups of cells (G) beneath the surface of the lesion.

H & E. x 350



observed that almost the entire parietal and visceral peritoneum of each animal was covered by firm, pink nodules varying up to 2.0 cm in diameter (Figure 72) and a lesser number of thin-walled, fluid-filled cysts up to 0.5 cm in diameter. In one case (number 232/233), lesions also involved the mediastinum, pericardium and parietal pleura, and the thorax contained approximately 50 ml of straw coloured fluid.

Histologically, lesions from sheep 217/218 consisted predominantly of aggregations of cells with large, clear, sometimes vesicular nuclei ranging up to 30μ in diameter. Nucleoli were large and frequently there were two per nucleus. There was abundant, eosinophilic cytoplasm and clearly defined cell boundaries (Figure 73). Mitotic figures were present but not numerous. On the surface of many nodules, and on the surrounding peritoneum, cells tended to form cords and small papilliform structures (Figure 74). Near the surface of some lesions, tubule-like structures were formed by slightly flattened cells.

In sheep 232/233, nodular lesions were found on histological examination to be papilliform structures with a central stromal "trunk" perpendicular to the peritoneal plane and with a tightly knit branch-like formation. Neoplastic cells about, and within, the stroma were arranged in single layers or groups (Figure 75). Cell nuclei were smaller than in sheep 217/218, generally about $9 \times 7\mu$, moderately dense-staining and most commonly with only one large nucleolus. Cytoplasm was abundant and mitotic figures were not common. Groups of neoplastic cells were seen within dilated diaphragmatic lymph vessels.

Lesions in sheep 264/265 appeared intermediate between those of the two previously described cases. In some places, cells were

predominantly in large clumps and had large, occasionally vesicular nuclei. Elsewhere, lesions were predominantly papilliform with abundant stroma. Cells of this neoplasm were seen to have involved lymphatics of the diaphragm, rumen and large intestine and had involved mesenteric and hepatic lymph nodes.

On the basis of the situation, histology and the absence of any other tumours in these animals, all three neoplasms were diagnosed as malignant mesotheliomas.

Methylcholanthrene, under the conditions of this experiment, resulted in deaths of some animals from severe liver injury after a minimum of six treatments (Table XXXI). In each sheep dying from the effects of methylcholanthrene there was 200 - 300 ml of serosanguinous fluid in both the abdominal and thoracic cavities. The livers of these sheep were enlarged, dark red and with fibrin deposits on their capsules. Histologically, there was severe centrilobular necrosis with pooling of blood in the centrilobular region.

The administration of N-methyl-N-nitro-N-nitrosoguanidine was ceased after it became apparent that intraperitoneal injections of this chemical were provoking a severe, sometimes fatal, fibrinous peritonitis in some sheep (Table XXXIV).

DISCUSSION

Mesotheliomas are rare in all species (Willis, 1952; Ackerman, 1954; Moulton, 1961) and have been reported in sheep only once (Plummer, 1956), apart from a probable pleural mesothelioma following

benzpyrene administration (de Kock 1958) (page 86). Willis (1952, 1960) considered the majority of reported mesotheliomas in humans were more likely metastatic lesions from unidentified primary carcinomas, and Keal (1960) did not rule out the possibility that peritoneal lesions encountered in asbestosis sufferers might have been metastases from very small tumours of the lung or ovary. Ackerman (1954), Hourihane (1964) and Evans (1966) considered that neoplasms arose only rarely from the peritoneum and stressed the importance of carefully differentiating such lesions from metastatic carcinomas. Amongst the domestic animals, mesotheliomas are said to arise most commonly in young calves (Moulton, 1961; Baskerville, 1967).

The mesotheliomas seen in sheep in this experiment resembled the most common form in man, although plaque-like lesions which spread centripetally have also been described (Ackerman, 1954; Evans, 1966). The relative uniformity of size and type of lesions encountered over the peritoneum of individual experimental sheep supports the opinion of Ackerman (1954) that this type of tumour is multicentric in origin. In humans, invasion of the underlying tissues is rare, although lymphatic permeation, as seen in these experimentally produced lesions, and metastasis to lymph nodes have been reported (Ackerman, 1954).

The histology of the three experimental ovine neoplasms recorded in this chapter was generally in accord with that described by Ackerman (1954) for human mesotheliomas. The absence of any other recognisable neoplastic lesions in these sheep further supports the diagnosis of mesothelioma in all three cases.

The incidence of neoplasia in sheep in this experiment was relatively low when compared with results reported following the

administration of the same carcinogens to laboratory animals.. This could have been due to the following factors :

Insufficient dose rate

In general, increased dose rates of carcinogens result in both increased tumour yield and decreased latent period for tumour development (Bryan and Shimkin, 1943; Andervont and Edwards, 1943; Berenblum and Haran-Ghera, 1957 a). In this regard, comparisons of results from different laboratories are difficult because dose rates in terms of body weight have often not been stated. However, the dose rates used in the present experiments were calculated to be well within, or toward the upper limit, of the range of dose rates commonly used in experimental animals. Wynder, Spranger and Fark (1960) demonstrated that, for skin carcinogenesis at least, the total dose of carcinogen applied, rather than frequency of application or duration of treatments, was the major factor influencing tumour formation. Most work with carcinogens in laboratory animals has involved constant intake or daily administration of the agent for lengthy periods, frequently the entire life of the experimental animals. The weekly administration of carcinogens in this experiment, for a maximum of 15 doses, reduced the relative total dose in comparison with many laboratory animal experiments.

The route of administration

The route of administration may affect the carcinogenic response of an animal although no reports of comprehensive studies of this aspect have been located. Most experiments have involved either cutaneous, subcutaneous or oral administration, which generally have resulted in more tumours of the skin, subcutaneous tissues and gastro-intestinal

tract respectively. In the present experiment, the intraperitoneal route was chosen for most animals because of ease of administration and because it would result in both local exposure of the peritoneum to high concentration of the chemicals used, and rapid absorption and circulation of these agents to other potentially susceptible tissues. Arcos, Argus and Wolf (1968) considered the intraperitoneal route should be satisfactory for the testing of carcinogens though it has seldom been used. The oral route of administration was not considered suitable for this experiment because of the difficulties in restraining sheep sufficiently to administer carcinogens without considerable risk of spillage on to both the administrator and the surroundings and because rumination of material administered by stomach tube could have resulted in contamination of pasture and working areas. There was also the possibility of degradation or alteration of these chemicals in the rumen of sheep.

The effects of the vehicle used for administration

The vehicle used for carcinogen administration may modify the effects of a carcinogen. Burrows, Hieger and Kennaway (1936) demonstrated that lard, which has been used as a solvent for agents being tested for carcinogenic properties, is itself slightly carcinogenic when administered subcutaneously to rats. Sheep sebum (lanoline) when used as a solvent, suppresses the carcinogenic effect of methylcholanthrene on the skin of mice (Simpson and Cramer, 1943, 1945). Because of variations in the chemical constitution of fats and oils of animal and vegetable origin and because of enhancing and inhibiting effects of solvents, Shimkin and Andervont (1940) and Leiter and Shear (1943) recommended that the synthetic, saturated compound tricaprylin be used as a standard solvent

for hydrocarbons. Arachis oil, used as a solvent for methylcholanthrene and benzpyrene in these experiments, is not noted as having any marked effects on the carcinogenicity of polycyclic hydrocarbons. More neoplasms develop when arachis oil is used as a solvent for the subcutaneous administration of benzpyrene to mice than when ox brain lipid or mouse fat is used (Dickens, 1947).

The implantation of crystals, fused cubes or threads impregnated with either methylcholanthrene or benzpyrene has been used to produce tumours of the alimentary tract (Stewart and Lorenz, 1941 b, 1942; Grant and Ivy, 1955; Zaldivar, 1964), mammary gland (Bonser, 1954) and central nervous system (Zimmerman and Arnold, 1943). Quantities of chemicals implanted by these techniques have been relatively small (in the order of 0.001 - 0.8 mg). The quantity (5 mg) of methylcholanthrene injected into the wall of the small intestine of each lamb in the present experiment was considerably in excess of the above amounts.

Urethane and nitrosoguanidine have previously been administered in aqueous solutions, as in this experiment, or in aqueous saline solutions.

Insufficient time between carcinogen administration and death

The latent period for the development of neoplasms varies not only with dosage schedules (Bryan and Shimkin, 1941) but also with the species (Fieser, 1938; Clayson, 1962). The latent period is related to the potential life span of the species, varying from a few months in mice (Fieser, 1938; Druckrey, Schmahl, Dischler and Schildbach, 1962) to many years in man (Goldblatt, 1947; Henry, 1947). Following carcinogen administration, animals should ideally be observed for

their entire life span to observe long term effects. Arcos et al. (1968) considered observations should be for an absolute minimum of one year, and generally for at least one third to one half of the average life span of the species. It was suggested by Clayson (1962) that rabbits used in carcinogen testing should be observed for at least 5 years and dogs for 8 - 10 years before it could be concluded that carcinogens had been without effect. The present experiment was necessarily terminated after approximately $3\frac{1}{2}$ years, a period which represents 50% of the usual life span of sheep prior to slaughter and approximately 33% of their potential life span.

Low susceptibility of sheep to the carcinogens used

A further possible reason for the relatively low number of tumours produced in this experiment might be that sheep are rather insusceptible to the effects of the carcinogens administered. Species variations in susceptibility to chemical carcinogens are well documented. Dogs are relatively insusceptible to carcinogenic hydrocarbons, monkeys are highly resistant to epithelial carcinogenesis by hydrocarbons (Pfeiffer and Allen, 1948) while guinea pigs are much more resistant to carcinogenic hydrocarbons than other commonly used species (Stenback, 1970).

The reason(s) for the small number of neoplasms produced in these experiments is not clear. The dose rate of carcinogen given at each treatment was comparable to the dose rate used in experiments with

laboratory animals but the total dose/Kg was relatively low due to the lesser number of treatments. The route of administration was different from those used in most laboratory animal experiments. It should, however, have resulted in exposure of most tissues to the carcinogens. The peritoneum of injected sheep must have been exposed to high concentrations of carcinogens. The relatively few neoplasms of the peritoneum developing in this experiment suggest that the cells of the peritoneum may be rather insusceptible to neoplasia. This is supported by the scarcity of naturally occurring mesotheliomas in all species.

Peanut (arachis) oil appears, from reports of its use, to be a satisfactory vehicle for the administration of polycyclic hydrocarbons and water has been used with success as the solvent for urethane and N-alkyl-N-nitroso-compound administrations. The most likely explanations for the relatively few neoplasms developing in the course of this investigation are either insufficient total dose of carcinogens, insufficient time between carcinogen administration and death, or that the sheep is a species relatively insusceptible to chemical carcinogens.

It is suggested that experiments using graded doses of methylcholanthrene and benzpyrene, administered subcutaneously in a tricapylin solvent to sheep, with similar absolute and per Kg doses of these chemicals being administered to rats and/or mice for comparison, might be the best method of establishing whether sheep are relatively refractory to the carcinogenic effects of these compounds.

SUMMARY

To investigate the susceptibility of sheep to chemical carcinogens, methylcholanthrene, benzpyrene, urethane and N-methyl-N-nitrosoguanidine were administered intraperitoneally to that species. In addition, one group received intra-mural injections of methylcholanthrene into the wall of the small intestine. The only resulting neoplasms were in three sheep to which benzpyrene had been administered. All were diagnosed as mesotheliomas.

Following discussion of a number of possible reasons for the relatively low incidence of neoplasms in the experimental sheep, it was concluded that the most likely explanations were either that the total doses of carcinogens administered were too low, the time from carcinogen administration till death was too short, or that sheep are relatively insusceptible to chemical carcinogens.

CHAPTER V

THE CARCINOGENICITY OF BRACKEN FERN

(Pteridium aquilinum var. esculentum)

INTRODUCTION

Bracken fern (Pteridium aquilinum) has been recognised as toxic to sheep, and more particularly to cattle, since at least the end of the nineteenth century (Penberthy, 1893; Storrar, 1893). The pathological changes associated with this toxicity in ruminants have been reviewed by Evans (1968), and include leucopenia, thrombocytopenia, increased capillary fragility, impaired blood clotting, and aplastic anaemia. Terminally there are widespread haemorrhages, particularly in the intestines. The radiomimetic nature of the bracken fern toxin has more recently been found to include neoplastic changes associated with chronic ingestion of bracken fern.

Prolonged feeding of bracken fern to cattle has resulted in "polypous tumourous" changes in the bladder mucosa, which were associated with chronic haematuria (Rosenberg and Heeschen, 1960). Extracts of urine from cattle being fed bracken fern have produced haemangioma-like lesions in the bladder of dogs, papilloma-like lesions when applied to the skin of mice (Georgiev, Vrigasov, Antanov and Dimitrov, 1963) and carcinomas of the bladder when introduced in cholesterol pellets to the bladder of mice (Pamukcu, Olson and Price, 1966). Cases of haematuria associated with neoplasia of the bladder were also

induced by Pamukcu, Goksoy and Price (1967) and Price and Pamukcu (1968) by prolonged experimental feeding of bracken fern to cattle.

Knowledge concerning the carcinogenicity of bracken fern was extended by Evans and Mason (1965) who produced carcinomas of the small intestine in all of 40 hooded rats fed bracken fern as 34% of their diet for 64 days. Mice fed this diet did not develop intestinal carcinomas, but all died as a result of pulmonary adenomas at between 12 and 19 months of age (Evans, 1968). Following experimental bracken feeding, intestinal carcinomas have developed in a high proportion of Japanese quail, one out of two sheep and one out of two hamsters. Of thirteen guinea pigs fed a supplement of fresh bracken fern ad lib. for 77 days, one developed an adenocarcinoma of the jejunum after 23 months, and when killed at 30 months the remainder had lesions of the urinary bladder mucosa ranging from epithelial hyperplasia to infiltrating carcinomas (Evans, 1968).

Bracken toxicity has not been widely reported in the literature from New Zealand, though the haemorrhagic syndrome has been recognised in both cattle and sheep (Hickman, 1910; Cunningham, 1952). Enzootic haematuria in association with undiagnosed "bladder lesions" were described by Kerrigan (1926) from an area of New Zealand where bracken fern was common. Smith and Beatson (1970) have since noted the occurrence of a range of urinary bladder neoplasms in cattle from a number of discrete geographic areas of New Zealand, apparently in association with access to bracken fern.

As native New Zealand bracken fern (Pteridium aquilinum var. esculentum) is a different variety from that in Britain and Europe (Pteridium aquilinum), and as Evans' experiment involving sheep was so

small, it was decided that the carcinogenicity of New Zealand bracken fern should be investigated. The experiments reported in this chapter were designed to investigate the carcinogenicity of bracken fern in New Zealand and its possible role in the production of "naturally" occurring small intestinal carcinomas in sheep.

MATERIALS AND METHODS

Rats : One hundred recently weaned rats of three strains and both sexes were divided into control and experimental groups as shown in Table XXXVI. Experimental feeding commenced in February and continued for 152 days, after which time a proprietary rat pellet feed was supplied to all groups until the experiment was terminated at 417 days. At this stage all surviving rats were killed and submitted to post mortem examination. Rats dying during the course of the experiment were subjected to a post mortem examination with particular attention being paid to the gastrointestinal tract and urinary bladder.

Sheep : Fifty female Romney lambs, approximately six months old, were divided into two equal groups, and respectively fed the bracken containing and control diets while housed indoors for 302 days. After this period, the sheep were grazed together on a predominantly ryegrass/white clover pasture until survivors were killed 140 - 150 weeks after the commencement of the experiment. All sheep dying during the course of this experiment or killed at its termination were examined post mortem with special attention being paid to the small intestine and urinary bladder.

T A B L E XXXVISurvival Times of Rats from the Commencement of Experimental Feeding

Strain	Sex	Treatment	Survival Time (Days)	Number surviving 417 days
Hooded	Male	Bracken fed	7, 10, 42, 194, 279, 397, 397, 405, 410	1
	Female	Bracken fed	224, 347, 348, 349, 350, 350	4
	Male	Control	242, 242, 276, 279, 279, 342, 349	3
	Female	Control	5, 9, 312, 373, 410	5
Sprague-Dawley	Male	Bracken fed	223, 299	8
	Female	Bracken fed	215	9
	Male	Control	192	9
	Female	Control	227*, 334*	8
Wistar	Male	Bracken fed		5
	Female	Bracken fed	3, 8, 12	2
	Male	Control	5, 222, 276	2
	Female	Control	2, 5, 9, 25, 58	0

* Mice with Lymphosarcoma.

Diet : The basic diet fed to control groups of rats and sheep consisted of 76% lucerne meal, 8% barley meal, 8% wheat screenings and either 8% meat meal or 8% milk powder. To this was added a proprietary mineral and vitamin premix to constitute 0.05% of the feed. Sodium molybdate and calcium sulphate supplementation according to the recommendations of Hogan, Money and Blayney (1968) was added to the sheep diet for the latter 200 days of their experimental feeding period.

The experimental bracken-containing diet consisted of 25% sun dried, chaffed bracken fronds and 75% of the above basic diet. Bracken fronds were harvested, dried and chaffed every 3 - 5 weeks from February to December 1968. Feeding of each batch of bracken commenced within 2 weeks of its being harvested.

Mixing of feed, and pelleting of sufficient quantities for the rats was carried out by a commercial firm.

RESULTS

Rats : Table XXXVI shows the times of death of control and bracken fern fed rats. Deaths during the first 60 days were attributed to cannibalism. The majority of later deaths were the result of bronchopneumonia. Two rats killed at the termination of the experiment (417 days) also had bronchopneumonic lesions.

No neoplasms were found in the rats fed bracken fern, but two female Sprague-Dawley rats fed the control diet and dying at 227 and 334 days respectively were found at necropsy to have lymphosarcomas.

Sheep : Between 80 and 310 days from the commencement of the experiment thirteen control animals, and seventeen of those receiving the bracken containing diet, died. Post mortem examination of all dead sheep revealed jaundice, and copper analyses indicated the cause of deaths was copper poisoning. The surviving twelve control animals and eight fed the bracken fern were killed and examined 140 - 150 weeks after the commencement of the experiment. No neoplasms were found in either group of animals.

DISCUSSION

Pteridium aquilinum var. esculentum fed to rats and sheep under the conditions of these experiments failed to produce neoplasms in either species. While the results of Evans and Mason (1965) and Evans (1968) indicate strain and species differences in susceptibility to the carcinogenic properties of Pteridium aquilinum, the strains of rats used in our experiments were chosen to include strains found susceptible by the above authors. The proportion of bracken fern in the experimental food in our experiments was smaller than that used in Evans and Mason's (1965) experiments (25% versus 34%), but the time over which it was fed to rats was greater (152 versus 64 days). Based on Evans and Mason's results, the period of observation of the rats in this experiment was in excess of the expected latent period for the development of neoplasms. The number of animals used was considered sufficient for an experiment of this type. Therefore, it appears that the New Zealand bracken fern fed to rats in this experiment was considerably less carcinogenic than the fern used by Evans and Mason.

The recognition of carcinomas of the bovine bladder in association with access to bracken fern in some parts of New Zealand (Smith and Beatson, 1970) indicates that New Zealand bracken fern in some locations is probably carcinogenic in the same manner as the European variety. The belief that bracken fern is most toxic when in the young frond stage has little experimental evidence to support it. Evans, Evans and Hughes (1954) fed October and June harvested bracken fern to cattle. Animals died after ingesting 464 - 604 lb of June harvested green fronds but showed no clinical signs following the ingestion of 200 lb of October harvested bracken containing little green material. This discrepancy could have been due to the different quantities of fern ingested, rather than to differences in toxicity. Schacham, Philp and Gowdey (1970) produced multiple adenomatous neoplasms of the ileum by feeding bracken fern to rats, whereas similar experiments the previous year, with fern collected at a different site did not result in neoplasia. They concluded that there were either geographic, seasonal or climatic effects altering the carcinogenicity of bracken fern. Field observations on seasonal patterns in the frequency of clinical and fatal bracken poisoning do not necessarily indicate fluctuations in bracken fern toxicity as the succulent, young, green fronds are grazed more than the mature form. Parker and McRae (1965) observed that sheep mainly ate bracken fern during July and August and that mortalities were most numerous during August to October. Observations on possible seasonal variations in carcinogenicity of bracken fern have not been reported. However, experiments reported by Evans, Barber, Jones and Leach (1969) indicate that the toxic and carcinogenic properties of bracken fern are probably contained in the one chemical molecule and, therefore, if there are geographic or seasonal variations

in one of these properties, variations in the other can also be expected.

The Cycad genus of ferns also possesses toxic and carcinogenic properties. In these ferns such properties depend on the hydrolysis of Methylazoxymethenol-D-glucoside to Methylazoxymethenol by - β glucosidase containing microorganisms in the intestinal tract (Laqueur and Spatz, 1968). A similar chemical conversion of the naturally occurring molecule may be required for Pteridium toxin to manifest itself (Evans et al., 1969).

From the above discussion, a number of possible reasons for the lack of development of neoplasms in the experiments described in this chapter present themselves :

- (1) If young green fronds are more carcinogenic, then the feeding of these throughout the experiment might have resulted in neoplasia. However, young fronds were included in the harvests of February, October and November and predominated in the December harvest. There was, therefore, a moderate exposure to this growth stage of bracken fern.
- (2) Seasonal or geographic conditions at the time and location of fern harvesting may have resulted in fronds with an unusually low carcinogenic activity.
- (3) It is possible that the flora of the gastrointestinal tracts of the rats and sheep in these experiments did not possess the enzymes necessary to produce a possible required chemical change in the bracken toxin.

- (4) There may be a difference in the amount or type of carcinogen produced in Pteridium aquilinum var. esculentum from that in Pteridium aquilinum, resulting in a lower carcinogenicity.

Evans et al. (1969) have made considerable progress in identifying the toxic and carcinogenic chemical in Pteridium aquilinum. They have isolated small quantities by thin layer chromatography, and established that it has a molecular weight of 156, an empirical formula of $C_7H_8O_4$, a carboxylic acid grouping and no benzene ring). When the chemical structure of the carcinogen has been defined, a more convenient experimental means of determining geographic, seasonal and variety differences in carcinogenicity of bracken fern will be available. Similarly, investigations of possible carcinogenicity in sheep will be more reliable when the quantity of toxin being administered can be monitored.

Because of the known carcinogenicity of bracken fern and its ability to induce small intestinal carcinomas in rats and Japanese quail, particular attention was paid to this aspect in the epidemiological study reported in Chapter III. Results of this study did not indicate any significant association between prevalence of small intestinal carcinomas and access to bracken fern. This supports the experimental evidence from feeding trials that bracken fern is unlikely to be responsible for small intestinal carcinomas in New Zealand sheep. However, for the various reasons discussed, more experimental work is indicated before these findings can be considered conclusive.

SUMMARY

To investigate the carcinogenicity of New Zealand bracken fern and its possible role in the aetiology of "naturally occurring" carcinomas of the small intestine of sheep, bracken fern harvested at 3 - 5 week intervals throughout the year was fed to three strains of rats and a group of sheep. Bracken fern constituted 25% of the diet of experimental animals and was fed to rats and sheep for 152 and 302 days respectively. A considerable number of deaths occurred in both species during the period of observation. Surviving rats were killed and necropsied 417 days after the experiment started and surviving sheep were similarly killed and examined at 140 - 150 weeks. No neoplasms were found in any of the animals fed the bracken containing diet.

Possible reasons for the lack of neoplasms in experimental animals are discussed, but no firm conclusions reached.

C H A P T E R V I

AN ATTEMPT TO TRANSMIT OVINE SMALL INTESTINAL

CARCINOMAS TO OTHER SHEEP

INTRODUCTION

Following the demonstrations by Ellerman and Bang (1908) and Rous (1910) that a form of chicken leukaemia and a chicken sarcoma respectively were transmissible by cell-free filtrates, a number of neoplasms have been shown to be similarly transmissible. In some of these, causal viruses have been identified and characterised.

There is evidence that a number of neoplasms of domestic animal species are transmissible. Amongst these are enzootic bovine leukosis (Bendixen, 1965), feline malignant lymphoma (Schneider et al., 1967; Theilen, 1968), feline fibrosarcoma (Gardner, Rongey, Arnstein, Estes, Sarma, Huebner and Rickard, 1970), mast cell neoplasms in dogs (Lombardi, Maloney and Rickard, 1963), nasal tumours of sheep (page 5), and sheep pulmonary adenomatosis (page 4). The presumed viral agents responsible for these neoplasms in domestic animals have not yet been identified and characterised, although a herpes-like virus has been isolated from ovine pulmonary adenomatosis lesions (page 4), and "C" type viruses have been demonstrated in lymphatic neoplasms of cattle and cats (Dutcher et al., 1967; Theilen, 1968) and in a feline fibrosarcoma (Gardner et al., 1970).

As one means of investigating the possibility that carcinomas of the small intestine of sheep are viral in origin, an experiment involving the inoculation of sheep with prepared suspensions of neoplastic tissue was instigated.

MATERIALS AND METHODS

Ten carcinomas of the small intestine were collected from ewes 10 - 15 minutes after slaughter by exsanguination. From each, approximately 1 gm of tissue from the mucosal - submucosal area was homogenised in a tissue grinder with 2 ml of Hank's balanced salt solution (H.B.S.S.) and placed in a 25 ml universal bottle in "dry ice" until transferred to a -40°C deep freeze $\frac{1}{2}$ - 2 hours later.

Bottles of tissue homogenate were thawed at room temperature seven days later, and the contents of each bottle made up to 10 ml with H.B.S.S. containing 500 i u/ml of streptomycin and penicillin. From each of five samples, 7 ml of tumour suspension were pooled (Pool I) and twenty 1.75 ml aliquots were dispensed from this pool and refrozen (-40°C). Portions of the other five homogenates were similarly pooled, dispensed and refrozen (Pool II).

The remaining 3 ml of each tumour homogenate were pooled and the resulting mixture was centrifuged at 7,000 g for 30 minutes. Following this, 10 ml of the supernatant fluid was dispensed into 5 x 2 ml aliquots (S.7000 g). The deposit was resuspended to make 10 ml with H.B.S.S. and dispensed to 5 x 2 ml aliquots while the remaining supernatant fluid was centrifuged at 45000 g for two hours. From the supernate after this centrifugation 10 ml was dispensed to

T A B L E XXXVII

Numbers of Lambs of Each Sex Treated with Various
Tumour Homogenate Preparations

Homogenate Preparation	Numbers of lambs	
	Males	Females
Pool I	5	5
Pool II	5	5
D.*7000 g	3	2
S.**7000 g	2	3
D.*45000 g	3	2
S.**45000 g	1	4

* Deposit from centrifugation at

** Supernatant fluid after centrifugation at

5 x 2 ml aliquots (S.45000 g) and the deposit was resuspended in 10 ml of H.B.S.S. and similarly dispensed (D. 45000 g).

All aliquots were refrozen to -40°C as soon as possible after dispensing. This meant they were thawed for times varying from an $\frac{1}{2}$ hour in the case of Pool I to 4 hours in the cases of samples S.45000 g and D.45000 g.

As newborn Romney x Cheviot lambs became available over the following 34 days, aliquots of tumour homogenate, or subsequent preparation, were thawed and injected into each animal 4 - 28 hours after birth. Of each aliquot, 1 ml was injected into the wall of the small intestine via a 25 gauge needle and the remaining quantity was left in the peritoneal cavity. The numbers of lambs of each sex which were treated with the various preparations are shown in Table XXXVII. The remaining 10 aliquots of each of Pools I and II were similarly administered to 20 castrated male Romney sheep one year old.

Tails were removed and male lambs castrated 3 - 6 weeks after birth. All experimental sheep were grazed on a predominantly ryegrass/white clover pasture and were drenched with an anthelmintic* four times in the first eight months of the experiment.

All animals were killed and submitted to necropsy 160 - 170 weeks after the commencement of the experiment.

RESULTS

Post mortem examination of all experimental animals inoculated with homogenised tumour tissue or its extracts failed to reveal any neoplastic lesions.

* Thibenzole, Merck, Sharp and Dohme, (N.Z.) Ltd.,

DISCUSSION

Experimental techniques previously used to transmit viral neoplasms have apparently been simple. In the majority, inoculation of filtered or centrifuged material, derived either from neoplastic tissue or from blood of affected animals, has resulted in the development of neoplasms in variable proportions of susceptible animals when given by an effective route. In this experiment, Pools I and II were crude suspensions of neoplastic tissue. The various centrifuged samples would have contained concentrations of cellular and subcellular particles of varying size, with the largest particles in sample D.7000 g. and smallest particles in sample S.45000 g. If free infective virus was present in the initial tumour homogenate, it should have been concentrated in the deposit at 45000 g (Cramer, 1964). Administration of infective virus to the wall of the small intestine should have given it ample opportunity to infect mucosal epithelial cells directly, and administration of part of each dose into the peritoneal cavity should have resulted in rapid absorption into the lymphatics, and hence to the blood stream, with dissemination to all susceptible tissues. The lack of development of neoplasms under the conditions of this experiment, however, does not necessarily exclude the possibility that the neoplasms in the donor animals were caused by a virus, nor does it indicate that such tumours are not transmissible, either horizontally (i.e. from sheep to sheep) or vertically (i.e. from dam to foetus or offspring).

Transmission of many viral neoplasms requires certain conditions which may vary between neoplasm types. To illustrate this, a number of factors which may cause the failure of attempts to transmit tumours

caused by viruses are discussed below : -

- (1) The inoculum, while containing infective virus, may contain insufficient virus to induce neoplasia.

It was demonstrated by Beard (1956, 1957) that plasma containing lesser numbers of avian myeloblastosis viral particles had a greatly reduced infectivity in inoculation tests. Doses of $4 - 10 \times 10^6$ particles were required to induce myeloblastosis in 50% of highly susceptible 3 day old birds, while up to 145×10^9 viral particles per dose were required to produce disease in 95% of injected chicks. Larger viral inocula induced visceral lymphomatosis in a greater proportion of birds than did smaller doses of virus (Burmester and Gentry, 1956).

The quantity of virus in Rous sarcoma lesions is dependent on the dose of virus initiating the neoplasm (Bryan, Calnan and Moloney, 1955; Bryan, 1956). When large numbers of viruses were administered experimentally, large quantities were present in the resulting neoplasm. Conversely, small viral doses resulted in either no neoplastic development or in sarcomas developing with a longer latent period and containing only small quantities of recoverable virus. "Spontaneous" sarcomas generally contain fewer viral particles than experimentally induced lesions, and this is thought related to their having been induced by relatively small numbers of viruses. It was considered by Gross (1961) that a similar low level of infective virus in "spontaneous" mouse leukaemia might have been one of the factors which made initial transmission of the neoplasms with cell-free filtrates difficult.

- (2) Virus induced neoplasms may contain little or no infective virus.

In initial experiments with the rabbit papilloma virus, Shope (1933) found that lesions induced with this agent in domestic rabbits were not transmissible. Shope (1960) subsequently demonstrated that infective virus disappeared from both cotton tail and domestic rabbits 8 hours after cutaneous inoculation and reappeared in the papillomatous lesions of the cotton tail rabbits but not in those of the domestic rabbits. The presence of viral antigens in papillomas of domestic rabbits was, however, detectable by serological means, and inoculation of other rabbits with material from non-infective papillomas protected those rabbits from subsequent infection. Noyes (1959) and Stone, Shope and Moore (1959) found that papilloma virus was demonstrable in the nuclei of keratinized cells near the surface of papillomas on cotton tail rabbits but not in actively proliferating cells. This virus apparently exists in two forms, one a "complete" infective form and the other an "immature" non-infective form. Apparently, viral particles do not mature to the infective form in domestic rabbits. A proportion of papillomatous lesions in both cotton tail and domestic rabbits change, in time, into squamous cell carcinomas, and in these, infective virus is not detectable in either strain of rabbit.

From lesions induced by the Rous chicken sarcoma virus, Carr (1944) found there was a maximum yield of infective virus in rapidly growing tumour tissue during the first 2 weeks of growth. Tumours examined beyond 40 days of growth yielded no virus.

Huebner, Lane, Welch, Calabresi, McCollum and Prusoff (1963) suppressed tumour induction in hamsters by adenoviruses by administering 5-iodo - 2-deoxyuridine at the same time as the virus was inoculated. As this compound prevents the replication of DNA viruses and as no virus replication was normally detectable following adenovirus infection, it was suggested that the effect of this compound was due to its preventing the incorporation of viral DNA into the genetic apparatus of the cell. This was supported by the work of Koproski, Jensen and Steplewski (1967), Sanbrook, Westphal, Srinivasan and Dulbecco (1968), Westphal and Dulbecco (1968) and Dulbecco (1969) which established that the DNA of polyoma virus in tissue culture is incorporated into cell nuclear DNA as part of the chromosomal chain. This incorporation may explain the disappearance of infective virus from some tumours caused by DNA viruses.

Viral particles were not seen in electron microscopic examinations of small intestinal carcinomas (page 34), suggesting that, if these neoplasms were caused by a virus, either complete viral particles were present in only small numbers or that virus existed in a "masked" form.

- (3) In especially susceptible hosts, some tumour viruses may destroy cells rather than initiate cell proliferation.

Shope rabbit fibroma virus inoculated in large doses into newborn rabbits may produce a generalised inflammatory disease (Duran - Reynolds, 1940b, 1945) and haemorrhagic lesions may result from the administration of Rous sarcoma virus to newly hatched

chickens (Duran-Reynals, 1940 a). Death from such effects may precede the development of neoplasms.

Inoculation of mouse embryo tissue cultures with Polyoma virus results in the destruction of approximately 80% of the cells, the remaining 20% of cells surviving in a transformed state (Rowson, 1966).

(4) The route of administration of the virus may be unsuitable.

The most effective route of administration of tumour viruses varies with the virus. Intravenous inoculation of avian erythroblastosis virus is considerably more effective than intra-muscular administration and 1400 times as effective as subcutaneous injection (Eckert, Beard and Beard, 1955).

Intraperitoneal inoculation of mouse mammary carcinoma virus is more effective than oral dosing (Gross, 1961), while intramedullary, intracranial, intraperitoneal and intravenous administrations of avian visceral lymphomatosis virus were all found equally effective by Burmester and Gentry (1954). Other routes, especially nasal, ocular, tracheal and oral were less effective but still resulted in lymphomatosis.

The Shope rabbit papilloma virus produces papillomas only if inoculated into the skin. If administered by other routes, tumours do not develop (Shope, 1933). Intratracheal inoculation of squirrel fibroma virus results in the development of lesions resembling those of pulmonary adenomatosis in other species (Kirschstein, Rabson and Kilham, 1958).

- (5) The age of animals at the time of inoculation may be a critical factor in viral carcinogenesis.

Gross (1961) reviewed earlier experiments in which he found that newborn (less than 16 hours old) C3H mice were susceptible to both parotid tumour (polyoma) virus and to leukaemia virus. The susceptibility of mice to polyoma virus decreased with every hour increase in age till the development of parotid tumours in mice inoculated when more than one day old was unusual. Susceptibility of mice to leukaemia virus also decreased with increasing age but less markedly so than to polyoma virus. Mice became insusceptible to leukaemia virus at 7 - 10 days of age.

The susceptibility of White leghorns to avian erythroblastosis was found to increase with age (Eckert et al., 1955) while in the case of mouse mammary carcinoma, age is a less critical factor. Older mice are less susceptible to mouse mammary carcinoma virus than suckling mice but adults can be infected by using larger viral doses (Bittner, 1956, 1957; Dmochowski, 1945; Andervont, 1945).

- (6) Breed, strain and individual genetic differences exist in the susceptibility of animals to tumour viruses.

An inbred "line 15" strain of White Leghorns is particularly susceptible to avian myeloblastosis and erythroblastosis, whereas commercial strains of White Leghorns are highly resistant to both diseases (Eckert, Waters, Burmester, Beard and Eward, 1954).

Similar strain differences in susceptibility exist amongst mice to mouse mammary carcinoma virus (Gross, 1961) and mouse leukaemia virus (Law, Dunn and Boyle, 1955).

- (7) The incidence of neoplasms in animals infected with tumour viruses can be influenced by dietary and other factors.

Observations by Siversten and Hastings (1938) and Tannenbaum and Silverstone (1953) that restriction of food (caloric) intake reduced the incidence of mammary carcinomas in infected mice were confirmed by White, White, Mider, Kelly and Heston (1944), who demonstrated that this effect also exerted itself on the development of "spontaneous" and carcinogen-induced leukaemia and hydrocarbon-induced sarcomas.

Altitude above sea level and environmental temperature may affect the susceptibility of mice to neoplasia induced by the leukaemic viruses (Mori - Chavez, 1958; Gross, 1960) and exposure of animals to chemical carcinogens appears to enhance the carcinogenic effects of tumour viruses (Rous and Kidd, 1938; Rogers and Rous, 1951; Ahlstrom and Andrewes, 1938; Salaman and Roe 1964).

- (8) Host immunity may prevent viral infection.

The host-tumour virus immunological response appears to be similar to that between animals and non-tumour-producing viruses.

The production of antibodies in response to infection by a number of tumour viruses has been demonstrated (Shope, 1933; Andervont and Bryan, 1944; Green, Moosey and Bittner, 1946; Eddy, Rowe, Hartley, Stewart and Huebner, 1958; Stewart, Eddy, Irwin and Lee, 1960; Rowe, Hartley, Law and Huebner, 1959; Gross, 1959), and for polyoma virus, at least, a protective effect has been demonstrated when introduced into virus free animals (Stewart, Eddy and Stanton, 1959).

Cross immunity between tumour viruses and other viruses may exist and, indeed, Shope (1932, 1936) demonstrated that recovery from infection with either rabbit fibroma virus or myxoma virus resulted in immunity to the other.

That maternal immunity to avian tumour viruses may be passed to chicks via the eggs was suggested when chickens from high tumour incidence flocks developed less disease than chicks from flocks free from lymphomatosis when experimentally infected with avian lymphomatosis virus (Gross, 1961). Transfer of maternal antibodies to litters of mice provided protection for litters of dams infected with polyoma virus (Law, Dawe, Rowe and Hartley, 1959).

A number of tumour viruses have been shown to stimulate interferon production and to be sensitive to its actions (Allison, 1963).

The development of carcinomas of the intestinal tract in sheep used in this experiment would have been good evidence that such neoplasms are transmissible, and most probably viral in origin. The lack of development of neoplasms in inoculated sheep, however, while being consistent with a non-infective aetiology, could be the result of viral "masking" in the neoplasms of the donor animal or because of suboptimal experimental conditions.

SUMMARY

To investigate whether small intestinal carcinomas of sheep were transmissible, forty new-born lambs, and twenty sheep one year old, were inoculated with either crude homogenates of neoplastic tissue or portions of such homogenates separated by centrifugation. Post mortem examination of all sheep 160 - 170 weeks after inoculation failed to reveal any neoplasms.

Possible reasons for lack of development of neoplasms in the experimental sheep were discussed, and it was concluded that the results of this experiment did not preclude the possibility that carcinomas of the small intestine of sheep might be viral in origin.

CHAPTER VII

GENERAL DISCUSSION

Results of the investigations reported in this thesis confirm earlier reports that carcinomas of the small intestine are common in sheep in New Zealand (Chapter III). Of particular interest was the close similarity in gross, microscopic and ultrastructural pathology of all the intestinal neoplasms examined, and their constant methods and directions of spread (Chapter II). This remarkable similarity of all carcinomas of the small intestine examined from sheep in the present series is in contrast to the reported variable nature of small intestinal carcinomas of man, and suggests the ovine lesions may represent a single pathological entity.

Although it has been shown that up to 1.5% of ewes slaughtered in New Zealand have small intestinal carcinomas, the cost of this condition to the sheep farmers and meat industry of the country can not be accurately calculated. If, however, it is assumed that 1% of slaughtered ewes have this disease, and that 80% of the 9 million "sheep" killed in New Zealand during the year ended September 1970 (Mills and Mackay, 1970) were ewes, then 90,000 slaughtered sheep, valued at approximately \$270,000, should have been condemned because of carcinomatosis. If a similar number of sheep die from the effects of intestinal carcinomas each year, then the total loss, including consequent loss of production, could be worth up to \$1,000,000.

Epidemiological investigations of small intestinal carcinomas in sheep (Chapter III) have indicated some factors possibly involved

in their aetiology, but it has not been possible to confirm an aetiological role for any of them. Experiments involving the administration of chemical carcinogens to sheep (Chapter IV), the feeding of bracken fern (Pteridium aquilinum var. esculentum) to rats and sheep (Chapter V), and the inoculation of homogenate preparations of neoplastic tissue into sheep (Chapter VI) did not result in the development of carcinomas. On the basis of the bracken feeding experiment and the results of the epidemiological investigation, it could be concluded that this plant was unlikely to be playing an important part in the aetiology of small intestinal carcinomas in New Zealand sheep.

People reporting on human small intestinal carcinomas have frequently conjectured as to the cause of these lesions, and reasons for their uneven distribution in the small intestine. Suggestions by Eichorst (1890) and Hoffman and Pack (1937) that duodenal ulceration might predispose to carcinomas were refuted by Mayo (1901), Hinton (1931) and Stewart and Leiber (1937). Several cases of carcinoma of the small intestine have been reported in association with chronic intestinal diseases, including non-specific granulomatous enteritis (Weingarten et al., 1959), regional enteritis (Ginzburg, Schneider, Dreizin and Levinson, 1956; Berman and Prior, 1964), atrophic jejunitis (Joske, 1960) and non-tropical sprue (Moertal and Hargraves, 1961). Of the above authors, only Joske (1960) and Berman and Prior (1964) (who found seven previous case reports of small intestinal adenocarcinomas in association with regional enteritis) considered the inflammatory lesion may have played a part in the pathogenesis of the neoplasm. Prigden et al. (1950) found no significant relationships between their cases and pre-existing typhoid fever, tuberculosis, chronic ulcerative colitis or adhesions. Wianko and MacKenzie (1936) considered

the incidence of small intestinal carcinomas increases nearer the ileocaecal valve, and that this might be due to delayed passage of contents through this valve, thus increasing the exposure of the more distal portions of the small intestine to carcinogens in the intestinal contents. It was suggested by Hoffman and Pack (1937) that the high proportion of duodenal carcinomas originating in the periampullary region might be attributed to the protrusion of the ampulla into the intestinal lumen, exposing it to eddying alkaline and acid currents and, therefore, to a greater irritation. The anatomic distribution of small intestinal carcinomas in sheep (Chapter II, Figure I), together with the lack of association of the condition with other intestinal lesions, indicates that the factors discussed above are not related to the ovine disease.

Two main categories of carcinogenic agents must be considered as possible causes of small intestinal carcinomas of sheep: chemical carcinogens and oncogenic viruses. While there are no features of individual neoplasms induced by either of these classes of agents which allow the immediate recognition of the cause from their morphology, a number of differences do exist in the action of these agents. Any one specific oncogenic virus (with the exception of polyoma virus) usually induces only one type of neoplasm in its host. Many chemical carcinogens on the other hand, induce a range of neoplasms in various organs and originating from different tissue types. As discussed by Alexander (1966), each chemically-induced neoplasm contains a tumour-specific antigen different from the tumour-specific antigen in any other neoplasm, including those neoplasms induced by the same chemical in the same organ in animals of the same inbred strain. Cross reactivity between tumour-specific antigens of chemically-induced

neoplasms has been demonstrated only rarely, and such cross reactivity has been weak. All neoplasms induced by a common oncogenic virus, on the other hand, contain a common tumour-specific antigen. This antigen is not related to the protein component of the virus, but results from viral infection of cells. Viral infection appears to stimulate the production of a new antigenic cellular component.

The uniformity of the pathology of small intestinal carcinomas of sheep (Chapter II), together with the relative scarcity of other neoplasms in New Zealand sheep and cattle (Chapter I), would be consistent with a viral aetiology for intestinal carcinomas. The demonstration of a common tumour-specific antigen in a number of these neoplasms would lend strong support to any suggestion that they are viral in origin. If different tumour-specific antigens were demonstrated in small intestinal carcinomas from different sheep, this would suggest the cause was a chemical carcinogen.

It has been considered that neoplasms in animals slaughtered for meat render such animals unfit for human consumption. Regulation 131, part 2, of the New Zealand Meat Regulations 1969, together with the Third Schedule to those regulations, stipulates that an inspector shall condemn as unfit for human consumption any carcass, together with all the viscera thereof, if the carcass or viscera is affected by, or exhibits lesions of "neoplasms, malignant with evidence of metastases". Justification for such action could be on either of two grounds : -

(a) that it is aesthetically unacceptable for people to consume the carcasses of animals with cancer, or (b) on technical grounds, in the belief that animals affected by neoplasia may act as a source of chemical carcinogen, or carcinogenic virus, likely to induce neoplasia

or other disease in man. Whether the condemnation of carcasses of sheep affected by small intestinal carcinomas can be justified on technical grounds will not be known until the cause of the disease is discovered and the distribution of the causative agent ascertained.

If these neoplasms are induced by a chemical carcinogen, it is probable that the doses of chemical responsible for inducing the neoplasms will have been metabolised long before the appearance of gross lesions. Results of investigations by Berenblum and Schoental (1942, 1943, 1946), Weigert and Mottram (1946 a, b) and Heidelberger and Weiss (1951) indicate that parenterally administered benzpyrene and its metabolic products are rapidly excreted. Although the carcinogenic metabolites of aromatic amines have not been identified, it has been demonstrated that metabolites are present at much greater concentrations in the urine than elsewhere (Clayson, 1966), suggesting that this compound is probably metabolised and excreted relatively quickly. The carcinogenic action of dialkyl nitrosamines is thought to depend on the conversion of nitrosamine to an alkylating agent which then reacts with cellular proteins or nucleic acids (Magee and Barnes, 1967). The carcinogenicity of most chemicals must depend on their entry into cellular metabolic processes, and on the chemical reactivity of either the administered chemical or its metabolites with either protein or nucleic acid cellular components. It is, therefore, unlikely that carcinogenic radicles would remain in an active form within an animal for the duration of the latent period for development of a neoplasm, the latent period generally being of the order of one tenth of the natural life span of the species (Page 97).

The presence of chemically-induced neoplasms in sheep from a particular farm would probably be indicative of continued exposure of all, or most, of the animals on that farm to the responsible agent. This being the case, if animal residues of chemical carcinogens present a human health hazard, animals with neoplasms probably present no greater risk than those without.

The handling or consumption of raw, or semi-cooked, meat from animals with viral neoplasms would present a human health risk only if the virus was pathogenic to man and infective virus was present in affected animals. The majority of oncogenic viruses so far investigated have proved to be species specific. However, Rous chicken sarcoma virus can infect newly hatched pheasants (Andrewes, 1932), ducklings (Duran-Reynals, 1942) turkeys and guinea fowl (Duran-Reynals 1943); mouse leukaemia virus may induce leukaemia if inoculated into newborn rats (Moloney, 1960; Gross, 1961) and polyoma virus will cause neoplasms in mice (Gross, 1953), hamsters (Eddy, Stewart, Young and Mider, 1958) and rats (Eddy, Stewart, Stanton and Marcotte, 1959). In addition, inoculation of blood from leukotic cows has resulted in the development of leukosis in sheep (Wittman and Urbanech, 1969) and a transmissible feline fibrosarcoma has been transmitted to dogs (Gardner et al., 1970). Even supposing carcinomas of the small intestine of sheep were caused by a virus capable of being pathogenic to man, and that the virus existed in affected sheep in an infective form, it is unlikely that ingestion of meat from affected animals would result in neoplasia, as infection of other than very young animals is difficult with most oncogenic viruses (Page 118). However, the possibility of ovine intestinal carcinomas being caused by an oncogenic virus which could also infect

man should not be ignored. If this were the case, sources of infection other than contact with, or ingestion of, meat might be important. The transmission of rabbit fibroma virus via flea and mosquito vectors has been demonstrated (Kilham and Woke, 1953) and any of the recognised means of virus transmission (e.g. aerosol spread, faecal excretion, urinary contamination, contact with, or ingestion of, cellular material including blood) must be considered possible sources of human infection.

On the basis of epidemiological investigations described in Chapter III, few possible sources of chemical carcinogens capable of producing intestinal carcinomas in sheep present themselves. However, if the geographic distribution of these neoplasms reflects a geographic distribution of a chemical carcinogen capable of affecting man, an uneven distribution of cancer incidence in man might be expected. No such uneven distribution has been reported, nor are we aware of any investigation into this possibility in New Zealand. Such an investigation, particularly with regard to carcinomas of the gastro-intestinal tract, seems worthwhile.

It is possible that a chemical carcinogen affecting sheep might not affect man. Species differences in susceptibility to chemical carcinogens are well documented and exposure of sheep to carcinogens, if contained in pasture and weed species, or fungi in the pasture, would be much greater than the exposure of humans to those agents. The possible formation of carcinogenic nitrosamines in some plant species (Magee and Barnes, 1967), or in the alimentary tract of sheep from ingested nitrates/nitrites and secondary amines (Lijinsky and Epstein, 1970), must be considered potential sources of carcinogens for grazing animals. The wide range of fungal species present in pastures and other feed-stuffs

is another potential cause of neoplasia. The majority of the fungi responsible for the mycotoxicoses listed by Ainsworth and Austwick (1959) were present on plant material and this is also true of Pithomyces chartarum, the cause of facial eczema (a toxicosis of the liver) in sheep and cattle in New Zealand. Aflatoxin, from Aspergillus flavus (Lancaster, Jenkins and Philp, 1961), the toxin of Penicillium islandicum (Miyake and Saito, 1965) and possibly the toxin of Penicillium griseofulvum (Barich, Schwarz and Barich, 1962; Hurst and Paget, 1963) are carcinogenic. It is possible that one, or more, from the wide range of fungi present in New Zealand pastures may produce a carcinogen responsible for the carcinomas encountered in slaughtered sheep. With the wide range of animal remedies and agricultural chemicals now available, many of which are cyclic and polycyclic hydrocarbons, the possibility that one or more of these might be carcinogenic must be borne in mind: DDT may be a liver carcinogen (Lancaster and Roe, 1964); arsenic produces neoplasms of the skin in man (Currie, 1947); and phenothiazine may possibly contain carcinogenic contaminants (Page 81). Exposure of sheep to most such agents would usually be in relatively small doses, and with such long intervals between exposures, that animal remedies or agricultural chemicals would require to be very potent carcinogens to produce significant numbers of neoplasms under usual farming situations. The results reported in Chapter III did not provide any indications that use of animal remedies or agricultural chemicals was resulting in the development of greater numbers of ovine small intestinal carcinomas.

S U M M A R Y

Carcinomas of the small intestine of sheep have been shown to be very uniform lesions. They are thought to arise from cells in the crypts of Lieberkuhn which, following malignant transformation, retain the ability to differentiate either to resemble columnar absorptive cells or to produce mucus. Primary lesions are most common about the middle of the length of the small intestine, although lesions are found along almost the entire length of the jejunum-ileum. Apparent multiple primary lesions were found in six of fifty consecutive sheep with carcinomas of the small intestine. The areas of mucosa in which carcinomas arise are replaced by neoplastic cells with a sparse to moderate collagenous stroma infiltrated by leukocytes, including eosinophils, and plasma cells. Those parts of lesions replacing the submucosa have a more abundant stroma, while lesions on the serosal surfaces of the abdominal and thoracic cavities consist, predominantly, of collagenous fibrous tissue with a relatively few neoplastic epithelial cells. Secondary lesions most commonly involve the mesenteric lymph node draining the primary lesion, the serosal surfaces of the small intestine proximal to the primary lesion, mesentery, omentum, parietal peritoneum and diaphragm. Cells which establish secondary lesions spread, mainly, via lymph vessels and the coelomic cavities.

Some neoplastic cells contain large, single, intracytoplasmic vacuoles lined by microvilli that resemble the brush border of normal intestinal epithelial cells. It is suggested that these structures may arise by invagination of the cell wall with subsequent closure, in a process similar to phagocytosis.

Examination of viscera of ewes slaughtered in seven geographic areas of New Zealand during 1967 and 1968 showed that there was a prevalence of carcinomas of the small intestine in British breeds of sheep of from 0.9% to 1.6% in different districts ($p < .01$). The prevalence in Merino, Merino Cross and Corriedale ewes was 0.2% and 0.3% in the two areas in which such ewes were inspected (difference between breed types, $p < .001$). Whether genetic or environmental differences were responsible for these variations in prevalence of carcinomas could not be determined. There is, however, a close correlation between sheep stocking rates in areas and the prevalence of carcinomas in ewes slaughtered from those areas.

Histories of over 22,000 ewes inspected for carcinomas of the small intestine during 1969 were obtained from vendors. Analysis of data concerning the physiographic, botanical and managerial factors these ewes had experienced on the vendors' properties revealed that there is a higher prevalence of carcinomas in ewes reported to have been exposed to crested dogtail, pastures on which greater quantities of potassic fertiliser had been used, or lower rainfall and ewes which had spent less than four years on the vendors' farms. On the basis of the available evidence no definite decisions could be made on the possible aetiological role of these factors.

To investigate the effects of known carcinogens in sheep, 20-methylcholanthrene, 3:4 benzpyrene, urethane and N-methyl-N-nitro-N-nitrosoguanidine were administered intraperitoneally to groups of sheep. The only resultant neoplasms were mesotheliomas in three out of seventeen sheep to which benzpyrene had been administered. From the relatively few neoplasms developing in experimental sheep it appears that either the treatment regimes were insufficient, the period

of observation following treatment was too short, or sheep are relatively insusceptible to the carcinogens used.

The carcinogenicity of New Zealand bracken fern (Pteridium aquilinum var. esculentum) was tested by feeding rats and sheep on a diet containing 25% bracken fronds. No neoplasms developed in animals receiving this bracken-containing diet. From this experiment, and from the epidemiological studies, it is concluded that it is unlikely that this plant is responsible for a significant proportion of carcinomas of the small intestine arising "naturally" in sheep in New Zealand.

An attempt to transmit ovine small intestinal carcinomas to other sheep, by inoculating sheep with various homogenates of neoplastic tissue, did not result in neoplasms in any of the experimental animals. It is not considered that this result precludes the possibility of these neoplasms being caused by an infectious agent.

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APPENDIX I

NAME:

VENDORS CODE NUMBER _____

ADDRESS:

DATE SHEEP KILLED	WHERE KILLED	NO. IN LINE	NO. INSPECTED	NO. OF TUMOURS
TOTAL				

(1) SOIL TYPE:

1	2	4	8	9	11	12	13	18	19
20	23	25	29	31	34	35	41	49	65
66	74	77	78	106	107	110	113	114	115
116	117	118	119	120	121	122	123	124	125
126	76	79	75	27	28				

OTHERS _____

(2) AVERAGE ANNUAL RAINFALL (INCHES):

< 40	41-60	61-80	81+
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(3) ANNUAL AVERAGE TEMP. (°F):

< 46	46.0-49.9	50.0-53.9	54+
------	-----------	-----------	-----

(4) AV. WINTER MIN. TEMP. (°F):

< 32	32.0-35.9	36.0-39.9	40+
------	-----------	-----------	-----

(5) AV. SUMMER MAX. TEMP. (°F):

< 65	65.0-68.9	69.0-71.9	72+
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(6) AVERAGE ALTITUDE OF FARM ABOVE SEA LEVEL (FT.):

< 200	200-499	500-1000	1000-1999	2000+
-------	---------	----------	-----------	-------

Appendix I contd.

(7) LATITUDE OF FARM ($^{\circ}$ S):

$< 30^{\circ}.30'$	$39^{\circ}.30'-40^{\circ}$	$40^{\circ}-40^{\circ}.30'$	$40^{\circ}.30'-41^{\circ}$	$>41^{\circ}$
--------------------	-----------------------------	-----------------------------	-----------------------------	---------------

(8) COMMON PASTURE SPECIES:

Ryegrass	Cocksfoot	Danthonia	Browntop	Dogstail
White Clover	Red Clover	Subterranean Clover	Lotus major	

OTHER _____

(9) PASTURE WEEDS:

	Never Seen	Unusual	Common
Bracken Fern			
Other Ferns			
Ragwort			

(10) LIST OTHER COMMON PASTURE WEEDS:(11) BREED OF EWES SLAUGHTERED:

Romney	Romney X (including Perendales)	Other British Breeds
Merino	Merino X	Corriedales

(12) AGE OF SHEEP SLAUGHTERED: (YEARS)

< 5	5	6	7	7+
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(13) REASON FOR SLAUGHTER:

Age	Toothwear	Poor Condition	Infertility
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OTHER _____

(14) SIZE OF FARM (ACRES) _____

< 100	100-499	500-1500	1500+
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Appendix I contd.

(15) NUMBER OF EWES FARMED: _____

< 200	200-499	500-1499	1500-4999	500+
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(16) TOTAL SHEEP CARRIED: _____(17) ∴ AVERAGE SHEEP STOCKING RATE/ACRE: _____

< 2	2-3	3-5	5-6	7+
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(18) ARE SHEEP MAINLY SET STOCKED OR MOB STOCKED:

Set	Equal	Mob
-----	-------	-----

(19) IF MOB STOCKED (OR EQUAL) AT WHAT DENSITY PER ACRE:
(ESTIMATE OF USUAL SITUATION)

<7	7-15	16-30	31+
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(20) TOTAL SUPER PHOSPHATE TOPDRESSING OVER LAST 7 YEARS:∴ AVERAGE RATE (CWT/ACRE) _____

Nil	< 10	10-25	25-50	50+	Don't Know
-----	------	-------	-------	-----	------------

(21) POTASH TOPDRESSING OVER LAST 7 YEARS:TOTAL QUANTITY: _____ CWT∴ AVERAGE RATE (CWT/ACRE) _____

Nil	< 4	> 4	Don't Know
-----	-----	-----	------------

(22) LIME TOPDRESSING OVER PAST 7 YEARS:TOTAL QUANTITY _____ CWT∴ AVERAGE RATE (CWT/ACRE) _____

Nil	< 10	> 10	Don't Know
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Appendix I contd.

(23) DDT USED OVER LAST 7 YEARS:

TOTAL QUANTITY : _____

∴ AV. RATE/ACRE _____ LB.

Nil	< 1.0	1.0 - 2.0	> 2.0+	Don't Know
-----	-------	-----------	--------	------------

(24) INSECTICIDES OTHER THAN DDT, APPLIED TO PASTURE
IN PAST 7 YEARS:

Nil	Fenonitrothion	Trichlorfon (Diptrex)	Diazinon
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OTHER _____

(25) SHEEP DIPS USED ON EWES SLAUGHTERED:

Diazinon	Ronnel	Luci-Jet	VC13	Asuntol	Delnav
Carbo-phenothion	Arsenic	Derris	Sulphur	Cresylic Acid	Parex
OTHERS _____					Don't Know

(26) WORM DRENCHES USED ON EWES SLAUGHTERED:

Nil	Phenothiazine	Thiabendazole	Nilverm	CCl ₄
Mintic	N.C.A.	Piperazine	Rametin	Neguvon
OTHERS _____				Don't Know

(27) SHEEP VACCINES USED ON EWES SLAUGHTERED:Clostridial

Nil	Once	Twice	2+	Don't Know
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(28) HAD THESE EWES HAD ACCESS TO STOCK LICKS?

No	A Little	Often	Don't Know
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WHAT TYPE? _____

- (29) HAD THESE EWES HAD SELENIUM ADMINISTERED TO THEM?

No	At Least Annually	Less Often	Don't Know
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- (30) WEEDKILLERS USED ON FARM IN LAST 7 YEARS:

Nil	Tordon	24D	245T	MCPA	MCPA	Gramoxone	Don't Know
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OTHERS _____

- (31) DISEASE ENCOUNTERED:

	Not Diagnosed	Unusual	Common
Facial Eczema			
Salmonellosis			

- (32) TYPE OF LIVESTOCK FARMING:

Sheep	Sheep + Beef	Beef	Dairy	Dairy + Sheep
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- (33) WERE MOST EWES SLAUGHTERED BORN ON YOUR PROPERTY?

Yes	No
-----	----

- (34) IF ANSWER TO (30) IS NO - HOW LONG WERE SHEEP ON YOUR PROPERTY?

_____ YEARS

4 yrs	4 yrs
-------	-------

- (35) IF ANSWER TO (34) IS LESS THAN 4 YEARS - FROM WHOM WERE THE SHEEP PURCHASED?

NAME :

ADDRESS :

WHEN?

(36) SUPPLEMENTARY FEED USED:

HAY

CHOUMOELLIER

ROOT CROPS

CEREAL GRAINS

APPENDIX II

SOIL TYPES ON FARMS IN SURVEY
AS INDICATED BY REFERENCE NUMBERS USED.

Number used on Questionnaire and in analyses	Soil Reference Number in Bulletin	Soil Types (Names)
1	1	Manawatu set
2	2	Opiki complex
8	8	Matapiro silt loam
9	9	Matapiro sandy loam
11	11	Raumai sandy loam
12	12	Ohakea loam
13	13	Tokomaru silt loam
	13 a.	Milson " "
	13 b.	Halcombe " "
	13 c.	Marton " "
18	18	Taupo sandy silt
23	23	Patea sand
	23 b.	Whananiki sand
25	25	Mangutea clay loam
29	29	Atua silt loam
31	31 d.	Purimu silt loam
66	66 d.	Westmere silt loam
77	77, 77 a, 77 b.	Matamau set
78	78, 78 a,	Kopua set
	78 b.	Dannevirke silt loam
106	106	Raumati silt loam
107	107	Makerua peaky loam
113	113	Bluff loam
114	114	Whangaehu loam
	114 a.	Turakina silt loam
115	115	Mahoenui silt loam
116	116 b.	Mangawahu silt loam

Appendix II contd

117	117, 117 a.	Moamahuki set
118	118 a.	Pohangina sandy loam
120	120	Mataikona silt loam
122	122	Makara stony loam
124	124	Ruahine stony silt loam
76	76 a.	Kiwitea loam
	76 b.	Kawhatau stony silt loam
	76 c.	Heretaunga silt loam
79	79 a.	Smedley loam
75	75, 75 a, 75 b.	Takapau set
27	27 c.	Waimarama sandy loam
28	28	Tinui silt loam and Waikura sandy loam.

APPENDIX III

WEED SPECIES NAMED ON LIST HANDED TO FARMERS

<u>COMMON NAME</u>	<u>BOTANICAL NAME</u>
Barberry	<i>Berberis vulgaris</i>
Barley Grass	<i>Hordeum bulbosum</i>
Blackberry	<i>Rubus fruticosus</i>
Broom	<i>Cytisus proliferus</i>
Buttercup	<i>Ranunculus</i> spp.
Capeweed	<i>Cryptostema calendula</i>
Chickweed	<i>Stellaria graminea</i>
Couch	<i>Agropyron repens</i>
Dandelion	<i>Taraxacum officinale</i>
Daisy	<i>Bellis perennis</i>
Dog Daisy (Ox-eye daisy)	<i>Chrysanthemum leucanthemum</i>
Flat Daisy	<i>Bellis perennis</i>
Dock	<i>Rumex</i> spp.
Fat Hen	<i>Chenopodium album</i>
Fennel	<i>Foeniculum vulgarae</i>
Fireweed	<i>Erechtites atkinsoniae</i>
Foxglove	<i>Digitalis purpurea</i>
Goats Rue	<i>Galega cfficinalis</i>
Gorse	<i>Ulex europacus</i>
Hawkweed	<i>Picris hieracioides</i>
Hemlock	<i>Conium maculatum</i>
Lambs Tongue	<i>Plantago</i> spp.
Linseed	<i>Linum marginale</i>
Lupin	<i>Lupinus arboreus</i>
Native bush	
Black Nightshade	<i>Solanum nigrum</i>
Penny Royal	<i>Mentha pulegium</i>
Plantain	<i>Plantago</i> spp.
Prickly Apple	<i>Datura</i> spp.

Appendix III Contd

Rats Tail	Sporobolus spp.
Red Root	Amaranthus cruentus
Rushes	Juncus spp.
Sedge	Cyperus spp.
Spurry	Spergula arvensis
Stinging Nettle	Urtica incisa
Storks Bill	Erodium cicutarium
Tall Fescue	Festuca arundinacea
Thistles - californian	Cirsium arvensis
- scotch	Cirsium lanceolatum (vulgare)
- swamp	Cirsium palustre
- variegated	Silybum marianum
- wing	Cirsium tenuiflorus
Trefoil	Medica and Lotus spp.
Water Cress	Nasturtium spp.
Wild Carrot	Daucus carota
Wild Turnip	Brassica campestris
Willow weed	Polygonum persicaria
Wire weed	Polygonum aviculare
Yarrow	Achillea millefolium
Yorkshire Fog	Holcus lanatus

APPENDIX IV

WEED SPECIES NAMED AS PRESENT ON SOME FARMS, THOUGH
NOT ON LIST HANDED TO FARMERS

<u>COMMON NAME</u>	<u>BOTANICAL NAME</u>
Land Cress (Twincreess)	Lantana camara
Mallow	Malva parviflora
Nodding thistle	Carduus nutans
Burr dock	Articum spp.
Ink Weed	Phytolacca octandra
Shepherd's Purse	Capsella bursa-pastoris