A STUDY OF A STRAIN OF ALBINO MICE WITH REGARD TO SUITABILITY FOR INVESTIGATIONS OF THE ROLE OF THE ADRENAL CORTEX IN MAMMARY GLAND GROWTH

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by

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P R E F A C E

The experiments reported in this thesis were carried out in the hope that they might assist in the clarification of the role of the endocrine system in the regulation of mammary gland development. A large part of the work was concerned with the elucidation of the general effects of adrenal insufficiency in mice, and with the maintenance of adrenalectomised mice by the injection of cortisol acetate. It is hoped that the results obtained with these mice will be of some assistance in future studies of the endocrine control of the growth of the mammary glands in mice, where it is dersired to exclude any influence mediated by, or originating from the animal's own adrenal cortex.

This study was prompted by Dr. D.S. Flux, to whom the author is indebted for guidance, encouragement, and patient instruction.

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CHAPTER I

INTRODUCTION

Scope of the Investigation

The role of the adrenal cortex in the growth of the mammary gland has been investigated by a number of workers, and the relevant studies have been most recently reviewed by Flux (1953, 1954) and by Folley (1955). The majority of published investigations utilised the rat as an experimental animal. The influence of adrenal steriods upon mammary development in the mouse (Flux, 1954) appeared to differ from that in the rat (Selye, 1954 a,b). Accordingly it was decided to extend the scope of previous investigations of the part played by the adrenal cortex in the growth of the mammary glands in the mouse.

Logically, any examination of the function of the adrenal cortical hormones in mammary growth should be preceded by an enquiry into the more general effects of alterations in the level of the normal spectrum of adrenal steroids. The greater part of this study is concerned with elucidation of the reaction of mice to bilateral adrenalectomy, and--in order that mammary growth could be studied in the adrenalectomised mouse---with the evaluation of the influence of certain factors upon the ability of the operated animal to survive. In Chapter V attention is directed towards the mammary gland. The experiment described, however, is to be regarded as preliminary; the final stage in the accumulation of information sufficient to allow the investigation of the effect of different factors on the mammary gland, without the complication of possible effects mediated by the adrenal cortex.

Presentation of Results

Where possible the results of experiments, and the details of statistical examinations of these data, are presented in tables removed from the text. The procedure followed is to include tables of mean values, together with the results of statistical tests, in the body of the thesis and to place tables of original data and detailed analyses of variance in the appendix. Tabular material is arranged so that it can be consulted in conjunction with the relevant portions of the text.

CHAPTER II

REVIEW OF THE LITERATURE

While there are a number of recent reviews dealing with the physiology of both the adrenal cortex and the mammary gland, these are broad in scope and do not, therefore, provide a detailed appraisal of the literature relevant to this study. Accordingly it was necessary to review certain aspects of these two fields of investigation in some detail. The pertinant literature has been considered in two sections:

(a) that dealing with the factors affecting the survival of adrenalectomised animals;

(b) that concerned with the role of the adrenal cortex in the development of the mammary gland.

A. FACTORS AFFECTING THE SURVIVAL OF ADRENALECTOMISED ANIMALS

The first studies involving adrenal removal were made by Brown-Sequard (1856). In the hundred years since this report, numerous investigations of the development of adrenal insufficiency, following adrenal ablation, have been reported. In the compilation of this review it was necessary to omit reference to much of the earlier published work on this subject. Detailed bibliographies are available in the earlier reviews by Britton (1930) and Grollman (1936) while later reviews by Swingle and Remington (1944), Parkes (1945), and Noble (1950, 1955) provide references to more recent studies.

The factors which affected the reaction of various animal species to adrenalectomy were widely investigated in the early part of the present century, and in 1933 Firor and Grollman suggested that the animal species, the age of the animal, the pre- and post-operative treatment, the anaesthetic and duration of

TABLE I

TABLE I.

MORTALITY RATES AND SURVIVAL PERIODS REPORTED WITH THE ADRENALECTOMISED RAT

Strain, Colony, Type of Operation[1]. and etc.	Sex	Age (days)	Weight (gm)	Total Number Operated	Surviving[2]. %	Dying within 48 hrs. %	Survival Period Range[3]. (days)	Survival Period Mean[4]. (days)	Reference
Sexually Immature Rats									
Long-Evans, untreated	M	30-32	50	34*	?	-	5-19	8.4	Blanchard & Tallman (1938)
Long-Evans, after treatment with cortin	M	30-32	50	500	4.0	-	5-19	8.5	.....
Wistar, untreated	M	28	50-60	192	2.6	-	-15	6.8	Cartland & Kuizenga (1936)
Wistar, after treatment with cortin	M	28	50-60	240	3.3	-	-15	7.2	.....
Wistar, Toronto colony, first series	M&F		35-80	93	4.3	?	-11	5.3**	Cleghorn, et al., (1936)
..... second series	M&F		40-60	34	3.0	-	3-14	8.3	.....
Wistar, Philadelphia colony	M&F	33	50-60	21	-	?	2-13	5.5**	.....
Wistar, Glaxo Lab. England	M&F	34	40-60	20	-	-	4-10	7.6	.....
Wistar, Connaught Lab., Univ. Toronto	M	26-28	40-60	20	-	-	5-14	10.9	.....
Hooded Norway	M&F	23	24-58	99	15.2	-	?	8.1	Cowie (1949a)
"Albino" (Wistar?) Two-stage operation	M&F	25	35-40	67	13.4	?	1-30	11.7**	Emery & Schwabe (1936)
.....	F	25-30		30	23.3	-	7-25	?	Schwabe & Emery (1939)
Wistar	M&F		-75	20*	?	80.0	1- 5	3.8	Firor & Grollman (1933)
Lewis colony	M&F	30		26	3.8	-	3-31	8.3	Gaunt, Gaunt & Tobin (1935)
Wistar	M&F	30		46	4.3	-	3-32	8.0	.....
.....	M&F	30		56	9.1	-	4-16	8.1	Marrazzi & Gaunt (1939)
Lister Institute	M&F		10-30	68	5.9	26.5	1-14	6.8	Gillman & Golberg (1942)
.....	M&F		31-80	141	0.7	2.8	2-13	5.9	.....
Inbred Albino Strain	M&F	28-35	-50	58	14-0	?	1-12	6.6**	Schultzer (1935)
.....	M&F	28-35	-50	77	-	-	3-12	5.7	.....
Strain not stated.	M&F	20-40		40*	?	-	3-16	6.8	Sisson & March (1935)
Sexually Mature Rats (months)									
Wistar, Toronto colony	M		80-200+	62	-	-	3-21	9.2	Cleghorn, et al., (1936)
.....	F		80-200+	52	11.5	-	3-30	13.1	.....
Hooded Norway	M	2		97	4.1	-	?	14.2	Cowie (1949a)
.....	F	2		102	10.8	-	?	14.3	.....
.....	M	4		72	9.7	-	?	16.2	.....
.....	F	4		59	6.8	-	?	19.2	.....
.....	M	12		32	12.5	-	?	13.8	.....
.....	F	12		79	6.3	-	?	17.9	.....
Wistar	M&F		100-	100*	?	13.0	1-21	8.9	Firor & Grollman (1933)
Wistar	M&F		148-236	24	12.5	?	2-30+	17.8***	Gaunt, Gaunt & Tobin (1935)
Lewis colony	M&F		148-236	37	64.9	-	14-	36.2***	.....
Lister Institute	M&F		110-120	33	3.0	-	4-14	8.4	Gillman & Golberg (1942)
Sprague-Dawley	M		180-182	84	-	-	7-18	11.3	Ingle, et al., (1943)
"Albino" (Wistar?) Two-stage operation	F	4-6		35	5.8	-	6-29	14.9	Kroc & Martin (1934)
..... Single operation	F	4-6		33	6.3	-	7-28	11.7	.....
"Piebald" strain	M&F		100-	60	-	?	2-18	8.2	Pencharz, et al., (1931)

1. Adrenalectomy a single stage operation unless otherwise noted.
2. The authors' various classifications of what constituted survival were followed.
3. Includes all animals dying whether from shock or from adrenal insufficiency
4. Excludes all animals dying within 48 hours. Means were recalculated to exclude these animals where necessary, provided that sufficient information was available.

* Adrenalectomised animals dying indefinite survival occurred but numbers of animals involved were not reported.

** Animals dying within 48 hours of the operation included in the mean.

***Animals possessing functional accessory tissue included in the mean.

anaesthesia, the operative technique, and the completeness of adrenal removal were influences of established importance. Subsequent workers (see Noble, 1950) have implicated the sex, reproductive state, and strain of the experimental animal.

The following classification, on the basis of mode of action, of the factors reputed to influence the mortality rates and survival periods of adrenalectomised animals was adopted for the material considered in this review:

(1) those that affected the degree of adrenal insufficiency achieved (Completeness of Adrenal Removal);

(2) those that influenced the operated animal, entirely or in part, independently of the state of adrenal insufficiency (Post-Operative Shock);

(3) those that affected the ability of the animal to withstand adrenal insufficiency (The Reaction to Adrenal Insufficiency).

1. Completeness of Adrenal Removal

> There is little doubt that removal of all functional cortical tissue is followed by death in nearly all species of animals, provided that the animal is not fed a diet high in salt or that it does not possess luteal tissue secreting progesterone (Noble, 1950).

Failure to obtain complete removal of all functional cortical tissue has been shown to be a cause of low mortality following adrenalectomy. In the intensively utilised rat, the wide variation in the mortality rates reported by different workers has been ascribed (Gaunt, 1933), at least in part, to the variable presence of functional cortical tissue after adrenalectomy. Of the less intensively investigated species, the rabbit, mouse and hamster resembled the rat, while the cat, dog, guinea pig and monkey have been uniformly reported to have high mortality rates after adrenalectomy (see later sections for references).

It was early recognised that, if adrenalectomy was to be uniformly successful, the integrity of the adrenal capsule had to be preserved during the operation. Indeed, Pencharz, Olmsted and Giragossintz (1931) implied that indefinite survivals after adrenalectomy reported by other workers (e.g. Lewis, 1923, Jaffe, 1926) were due entirely to failure to ensure that the adrenals were removed with the capsules intact. However, the results of Gaunt and his colleagues (Gaunt, 1933; Gaunt, Gaunt and Tobin, 1935) indicated clearly that functional cortical tissue could exist apart from the principal glands.

There appeared to be considerable variation, in different strains of rat, in the distribution of accessory tissues in relation to the chief glands. Schultzer (1935) reported that removal of the pedicle, a portion of the kidney capsule, and the peri-adrenal fat with the adrenal led to increased mortality, when compared with the removal of the gland alone. Gaunt (1933) failed to show such a difference.

The rapidity with which accessories assumed functional importance was implicated as an additional source of variation by the results of Gaunt and his co-workers. The administration of cortical extract (Gaunt and Gaunt, 1934), or the provision of saline drinking fluid (Gaunt, Tobin and Gaunt, 1935) resulted in a greatly increased proportion of indefinite survivals. Whereas less than half the treated animals died on discontinuation of the treatment, 95 per cent of their untreated litter mates succumbed. A period of therapy with cortical extract was, however, without effect on the mortality rate of adrenalectomised rats of similar (Wistar) origin studied by Blanchard and Tallman (1938) and by Cartland and Kuizenga (1936).

In some strains of rat the relatively longer survival period of the mature adrenalectomised animal led to a greater proportion of indefinite survivals

than that characteristic of immature rats of the same strain. Gaunt, Gaunt and Tobin (1935) compared the effect of age in their own strain and in the "Lewis strain" (Lewis, 1923). When adrenalectomised at 30 days of age approximately 95 per cent of the animals in each strain died whereas when operated at the adult stage 35 per cent of the animals of the "Lewis strain" died while the mortality rate of animals of the "Gaunt strain" was reduced to 87 per cent.

The mere possession of accessory cortical tissue did not necessarily allow indefinite survival, and it has been further shown that animals possessing functional accessory (or residual) tissues may eventually die. Wyman (1928) and Gaunt, Potts and Loomis (1938) have described a state of chronic adrenal insufficiency in adrenalectomised rats possessing histologically demonstrable accessory cortical tissue. The majority of such animals eventually died. Pencharz et al. (1931) reported the development of a similar state of chronic insufficiency in some of a group of adrenalectomised rats, in which small fragments of the chief adrenal glands had been left at the operative site. Gaunt (1933) suggested that any untreated adrenalectomised animal surviving longer than 21 days post-operatively should be regarded as possessing functional cortical tissue, even though such an animal eventually died.

It has been emphasised (e.g. Gaunt, 1933; Cowie, 1949a) that failure, even after a rigorous macroscopic search and histological examination of all suspicious tissues, to demonstrate cortical tissue in indefinite survivals did not imply that such tissue was not present. The only well supported test of the completeness of adrenal ablation was the death (within three weeks of adrenalectomy) of the operated animal. A number of other tests, involving the ability of the animal to withstand shock, have been proposed (e.g. Marder, 1950). These tests were of doubtful value since it had been shown that animals possessing

fragments of functional cortical tissue, sufficient for existence in a neutral environment, could not withstand stress (Brogi, 1954; Wyman and Tum Suden, 1929).

It is not always possible to conduct a survival test and there is need, therefore, for an index of adrenal cortical function which will detect supposedly adrenalectomised animals possessing functional cortical tissue. Speirs and Meyer (1949) have claimed that the degree of eosinopenia resulting from the injection of adrenaline (or adrenocorticotrophin) can be used to distinguish completely adrenalectomised mice from those possessing a minute amount of functional cortical tissue. The possibilities of this technique have not as yet been adequately investigated.

2. Post-Operative Shock

Death, the end-point of survival after adrenalectomy, would seem to be conclusive and unambiguous. This, however, is not the case, since in adrenalectomised animals death may result from causes remote from, and seemingly unconnected with, adrenal insufficiency. These causes, the sum of the effects of operative interference and detrimental environmental conditions, can be referred to collectively as post-operative shock. The importance of post-operative shock has been emphasised recently by Noble (1950):

> ...in many of the older experiments in which death followed rapidly after adrenal removal, the severity of the operative procedure and the various detrimental environmental conditions were often underestimated as factors causing mortality.

A number of the factors contributing to post-operative shock have been identified. The period of anaesthesia (Firor and Grollman, 1933) and the type of anaesthetic employed (Firor and Grollman, 1933; Nissim, 1953) have been implicated in the rat and mouse. In the dog the contribution of damage

to the splanchic ganglia (Freud, Uyldert and Waterman, 1938) has been emphasised. The improvement, with practice, in the results obtained by a number of investigators (e.g. Pfeiffer and Hooker, 1940; Rogoff and Stewart, 1926, 1928-b) indicated that non-specific trauma could be a major component of total post-operative shock. The care and condition of the animal prior to operation (Cleghorn, Cleghorn, Forster and McVicar, 1936; Firor and Grollman, 1933; Jaffe, 1926) and exposure to extreme environmental temperatures after adrenalectomy (Weiser and Norris, 1936; Wyman and Tum Suden, 1929) have been shown to affect the survival times of adrenalectomised rats. While stress prior to the operation could have affected the development of adrenal insufficiency (by causing a depletion of cortical hormone), the major effect of post-operative stress appeared to be reflected in the degree of post-operative shock (Gillman and Golberg, 1942).

There have been but few attempts to define the quantitative influence of post-operative shock. The majority of workers have, however, attempted to minimise its effect by adopting techniques which allowed rapid removal of the adrenals, with minimal damage to other structures, and have endeavoured to maintain optimal pre- and post-operative conditions.

A reduction in the level of post-operative shock--reflected in the reduced occurrence of short survival periods--was obtained by early workers (Banting and Gairns, 1926; Elliott, 1914; Rogoff and Stewart, 1926, 1929; Zwemer, 1927b) upon adoption of a two stage operation, in which a recovery interval of a week or more was allowed to elapse between the removal of the first and second glands. Claims in favour of the double operation have been advanced for a number of species, including the rat, generally regarded as the most easily operable of laboratory animals (e.g. Cramer and Horning, 1939 (mice); Gaunt and Hays, 1938 (ferrets);

Martin, 1932 (rats)). Not all workers, however, have found it necessary to resort to two stage operations, and even in the dog, in which the operation was held (Britton, 1930) to be more difficult than in most laboratory species, the single stage operation has been successfully employed (Cleghorn, Armstrong and Austen, 1939).

Other authors have attempted to establish criteria whereby animals dying from the effects of shock could be distinguished from those dying as a result of the development of adrenal insufficiency. Early investigators employing the dog and cat placed considerable reliance upon the occurrence of atypical symptoms and post-mortem conditions (Britton, 1930), in deciding whether short-lived adrenalectomised animals had died as a result of post-operative shock. Later workers indicated that this reliance was probably misplaced (Freud, et al., 1938; Cleghorn, et al., 1939), and preferred to maintain the operated animals by some form of replacement therapy, during the period when operative shock was expected to have its effect (Allers and Kendall, 1937; Cleghorn, et al., 1939).

In the rat, Gillman and Golberg (1942) suggested, as a result of comparison of adrenalectomised and control-operated rats, that the effects of post-operative shock could be ignored, provided that animals dying within 48 hours of the operation were not considered. This hypothesis has been accepted by subsequent investigators (e.g. Cowie, 1949a). While the procedure could have eliminated the immediate effect of surgical interference, it did not allow for any prolonged effect of post-operative shock. Because this factor was a cause of death, in control-operated animals, only during the first 48 hours it could not be assumed that it had not contributed to death in adrenalectomised animals over a longer period.

Firor and Grollman (1933) asserted that the total effect of operative procedure could be evaluated by the comparison of the survival times of two groups of adrenalectomised rats, where one of these had received a short period of "efficacious therapeutic treatment". These authors claimed that, in their strain of rat, operative interference reduced the mean survival period of the adrenalectomised animal by approximately two days. This claim was based on small numbers and was not supported by estimates of variability. It was not confirmed by the results of Cartland and Kuizenga (1936) and Blanchard and Tallman (1938) both of whom found no differences between treated and untreated animals. If the effect claimed by Firor and Grollman was real (in the absence of estimates of variance the reality of the effect could not be assessed) then the divergence from the results of later investigators could have resulted from a marked difference in the occurrence of very short survivals. In other experiments Firor and Grollman reported a high proportion of survivals of less than 48 hours in untreated adrenalectomised rats. These were not excluded in the computation of mean survival times. It was not clear how they regarded such short survivals in the comparison of treated and untreated rats. The results presented by Blanchard and Tallman (1938) were based on over 400 immature rats and the incidence of survival periods of less than 48 hours was negligible. It could, therefore, be safely concluded, provided the incidence of short survivals was small, that the exclusion of animals dying in less than 48 hours eliminated any effect of post-operative shock. Although direct comparisons have not been made in other species, it appeared that the survival times reported by Rogoff and Stewart (1928b) in their later series of experiments with the dog were not inferior to those obtained by other authors

(e.g. Harrop and Weinstein, 1933) after administration of extracts of the adrenal cortex.

3. Factors Affecting the Reaction to Adrenal Insufficiency

(a) Species and Strain

(i) The Rat. Numerous studies of the effects of adrenal excision have been conducted with the rat, and mortality rates ranging from 100 per cent (e.g. Schultzer, 1936) to less than 20 per cent (e.g. Lewis, 1923) have been reported. It gradually became apparent that these inconsistencies did not necessarily indicate wide variations between strains and colonies of rats in tolerance of total adrenal insufficiency. Variation in the intake of sodium chloride (Cleghorn, et al., 1936), incompleteness of adrenal removal (Gaunt, 1933), and in the degree of post-operative shock (Gillman and Golberg, 1942) have all contributed to the wide differences in mortality rates reported by the various authors.

The results obtained by a number of investigators have been summarised in Table I. The effects of dietary intake of salt, reproductive state, presence of functional cortical tissue and post-operative shock on the survival period have been excluded from the means and ranges tabulated. Despite the great variety of surgical procedures employed, the considerable variation in age, and the diverse origin of the operated animals the majority of the estimates of the mean survival period for the rat were between 5 and 9 days. The considerable variation in the individual survival periods recorded by the majority of workers emphasised the inadequacies of estimates of population differences based on small numbers of animals.

(ii) The Mouse. The operation in the mouse has generally been considered more difficult than in the rat (Firor and Grollman, 1933). Although this species seemed, like the rat, to be relatively resistant to the effects of trauma (Dorfman, Shipley, Schiller and Horwitt, 1946), the operation was complicated by the size of the experimental subject and by the close association of the right adrenal with the vena cava (Firor and Grollman, 1933).

There have been few detailed published studies of the reaction of untreated mice to adrenalectomy. The earliest report was that due to Masui (1928). This worker found that 23 per cent of seventy-two bilaterally adrenalectomised mice of mixed ages survived indefinitely. Deaths occurred up to the twelfth post-operative day and the average survival period was approximately 6 days. A similar mean survival time of 6.7 days was reported by Firor and Grollman (1933) for nine albino mice, adrenalectomised at adult ages, which died between the third and eleventh post-operative day. A further eight mice survived adrenalectomy for less than one day while three survived indefinitely. Accessory cortical tissue of macroscopic size was detected in these latter mice. Howard (1937, 1946) recorded total mortality, within twelve days of adrenalectomy, in eighteen mice operated at 21 days of age. The mean survival period was not given, but the mode of the distribution of deaths was 4 days. A mortality rate of less than 50 per cent was found by Cramer and Horning (1939) after adrenalectomy (in two stages) in twenty-six mature mice of mixed strains. Cortical tissue was detected macroscopically in two of fifteen indefinite survivors. Dorfman, et al. (1946) adrenalectomised ninety-two mature male mice of an albino variety and obtained a mortality rate, by the twentieth post-operative day, of 83 per cent. Accessory cortical tissue was not detected in any indefinite survivor.

A strain difference in the response to administration of sodium chloride was suggested by the results of Marder (1950) and Pfeiffer and Hooker (1940). While individuals of the CBA strain responded to increased dietary sodium chloride neither the A strain nor the C57 Black strain showed an extension of survival as a result of alterations of the diet.

(iii) The Dog and Cat. Mean survival periods of 5 to 10 days have been reported for the untreated adrenalectomised dog (Banting and Gairns, 1926; Rogoff and Stewart, 1926-1928). The untreated adrenalectomised cat has been reported to have mean survival times of 5 to 11 days (Britton, 1931; Elliott, 1914; Hartman, Brownell and Hartman, 1930; Marine and Baumann, 1927; Rogoff and Stewart, 1929; Zwemer, 1934). In neither species has accessory cortical tissue been found a cause of indefinite survival, though accessory cortical bodies were detected at adrenalectomy in 6 per cent of cats by Rogoff and Stewart (1929).

(iv) Other Species. A number of animals of other species have been adrenalectomised and details of the reaction of the rabbit, the guinea-pig and the monkey to bilateral adrenalectomy have been given in the reviews by Britton (1930), Grollman (1936), and Noble (1950) while the effect of the operation in the hamster, the sheep, the goat and in man has been summarised by Noble (1955).

(b) Age at Adrenalectomy

The influence of age at adrenalectomy on the death rate following the operation has been described in a previous section. It was concluded (Gaunt, et al., 1935) that the lower mortality in the older rat was caused by a greater degree of hypertrophy of accessory cortical tissue.

Age at the time of operation has also been shown to affect the period for which an animal will survive after adrenalectomy. The majority of investigations of this effect of age have been carried out on the rat.

Firor and Grollman (1933) classified 157 adrenalectomised rats of both sexes according to body weight at operation, and claimed an overall positive relation between age (as measured by weight) and survival time. This conclusion has been questioned by Gillman and Golberg (1942) on the ground that the means presented included data from animals surviving less than 48 hours after the operation. When, however, the means for the different groups were recalculated with the data from these short-lived animals excluded (see Table I), the differences between the means were largely of the same order as those published by Firor and Grollman. The largest difference was that between the means for the groups on either side of puberty. The reality of other smaller differences was, in the absence of estimates of variability, questionable. The results of Sisson and March (1935) on a smaller group of rats of both sexes revealed a similar marked increase in the survival period at puberty.

The most comprehensive study of the influence of age has been provided by Cowie (1949a). Over 300 rats of each sex were adrenalectomised at one of five ages which extended from 21 days to 365 days. Here again the most remarkable difference was related to puberty, but a stepwise increase in the survival period with age after puberty was also indicated. (The results of this worker are reproduced graphically in Fig. 4).

In the hamster, Snyder and Wyman (1951) found sexually mature females survived significantly longer than immature females following adrenalectomy. The male hamster did not exhibit a corresponding age-increase in survival time.

(c) Sexual Condition

While it has been conclusively shown that adequate levels of progesterone, of either endogenous or exogenous origin, can promote survival of adrenalectomised animals, the influence of other gonadal hormones, and the survival value of the testis and non-luteinised ovary have not been clearly established.

(i) Sex and Gonadectomy. The period for which female rats survive adrenalectomy has been variously reported to exceed (Cowie, 1949a; Kroc, 1938; Sisson and March, 1933), to fall short of (McKeown and Spurrel, 1940), or not to differ significantly from (Cleghorn, et al., 1936; Gaunt, 1933; Schultzer, 1935) that of the male.

With rats of the Sprague-Dawley strain, Kroc (1938) reported that adrenalectomised females survived slightly longer (two days) than males. This difference, on the borderline of statistical significance ($p < 0.06$) appeared to result from a depressing effect of the testes on survival and not from a favourable effect of the ovary. In a study involving more than 600 rats of the hooded Norway strain, Cowie (1949a) showed conclusively that, at a number of mature ages, adrenalectomised female rats outlived simultaneously operated males. This difference was not apparent in rats operated prior to puberty. McKeown and Spurrel (1940) reported that the mean survival period of fifteen male albino rats, adrenalectomised at body weights between 130 and 150 g., was slightly, but significantly, greater than the mean survival time of a similar group of female rats.

Ovariectomy was found by Kroc (1938) to be without effect on the period for which female rats of the Sprague-Dawley strain survived adrenalectomy. Mendez (1934) detected a slight, doubtfully significant, amelioration of the effects of adrenal insufficiency in the immature male rat following gonadectomy while Kroc (1938) found that castrate mature male rats survived adrenalectomy on average three days longer than non-castrate males, but this difference was rendered non-

significant by the increased variability in the castrate group. On the other hand, Gley, Deygard and Ripaille (1947) claimed that castration led to a reduction in the survival time of adrenalectomised male rats.

Similar survival periods for mature female mice of the CHI and Swiss albino strains and mature males of the CHI strain were reported by Hill (1948). A shorter survival period was obtained in ovariectomised-adrenalectomised mice of the albino strain, but the author discounted any effect of the ovary because of the small numbers involved in the comparison of ovariectomised (nineteen mice) and non-ovariectomised (eleven mice) animals. Cramer and Horning (1939) claimed that mature females, of mixed strains of mice, outlived mature males after adrenalectomy. The reality of this effect was hard to assess, both because of the small numbers involved in the comparison and because of the undetermined influence of accessory cortices, the presence of which was suggested by a peponderance of prolonged survivals.

The influence of sex and gonadectomy has been comprehensively investigated in the hamster by Snyder and Wyman (1951). Female hamsters survived significantly longer after adrenalectomy than males, and this difference was related to the number and functional state of corpora lutea in the ovaries of the female. Rogoff and Stewart (1926, 1929) reported survival periods for adrenalectomised male and female dogs and cats which did not show any effect of sex.

(ii) Pregnancy and Pseudopregnancy. The presence of functional corpora lutea, during pregnancy and pseudopregnancy, has been found to promote the survival of adrenalectomised rats (Firor and Grollman, 1933; McKeown and Spurrell, 1940), cats (Collings, 1941), dogs (Rogoff and Stewart, 1927, 1928c; Swingle, Parkins, Taylor, Hays and Morrell, 1937) and ferrets (Gaunt and Hays, 1938). Parkes (1945) suggested that failure to demonstrate an effect of pregnancy in the cat, when operations were carried out about mid-term (Corey, 1928; Rogoff and Stewart,

1929), was due to the low level of activity of the waning corpora lutea.

Pfeiffer and Hooker (1940) could not show an effect of pregnancy or pseudopregnancy in the mouse, however, administration of gonadotrophins was effective in prolonging survival in this species. It was suggested (Pfeiffer and Hooker) that the level of activity of luteal tissue in the pregnant mouse was too low to allow survival after adrenalectomy. A similar effect of gonadotrophic stimulation in the rat was shown to be due to luteinisation of the ovary (D'Amour and D'Amour, 1939; Emery and Greco, 1940).

(iii) Administration of Gonadal Hormones. Evidence that progesterone would effectively prolong the survival period of adrenalectomised animals has been provided by a number of investigators (Parkes, 1945). Earlier reports that progesterone was without effect (Cavanaugh and Gaunt, 1937; Emery and Schwabe, 1936; Swingle, et al., 1937) probably resulted from the administration of the steroid at sub-threshold levels (Parkes, 1945).

Minimal dosages of progesterone between 0.5 and 4 mg. daily have been reported to delay or prevent death for 15 to 20 days in the adrenalectomised rat (Bourne, 1939; Emery and Greco, 1940; Gaunt, Nelson and Loomis, 1938; Greene, Wells and Ivy, 1939; Schwabe and Emery, 1939; Wells and Greene, 1939). In the ferret the minimum daily level of progesterone which maintained the adrenalectomised animal in good health was 2 mg. (Gaunt and Hays, 1938). Adrenalectomised mice were protected by 1 mg. daily while about 50 per cent survived when injected with 0.5 mg. of progesterone daily. 0.5 mg. of progesterone daily would allow approximately normal growth in the adrenalectomised hamster (Snyder and Wyman, 1951).

Progesterone has been compared with desoxycorticosterone acetate (DCA) in a number of species. The two hormones have been reported to be of equal

potency as regards ability to promote survival in the adrenalectomised rat (Emery and Greco, 1940). In the ferret DCA was about three times as effective as progesterone in allowing survival (Gaunt and Hays, 1938) while in the hamster progesterone was almost three times as potent as DCA (Snyder and Wyman, 1951).

The effect of oestrogens upon the survival of adrenalectomised animals is not clear, but it does not appear to be benefitial. Small doses of extracts rich in oestrogens (Emery and Schwabe, 1936; Swingle, et al., 1937), or of oestradiol and synthetic oestrogens (Wells and Greene, 1939) were without detectable effect on adrenalectomised rats. On the other hand, Gaunt, Potts and Loomis (1938) found that oestrone was toxic to the adrenalectomised rat and Pfeiffer and Hooker (1940) reported oestradiol toxicity in adrenalectomised mice. During the prolonged oestrous period of the ferrect, Gaunt and Hays (1938) found that the level of adrenal cortex extract needed to maintain the adrenalectomised animal was increased two or three fold.

Androgens have been found without effect, even when given at considerable levels, on the survival of the adrenalectomised rat (Spurr and Kochakian, 1939; Wells and Greene, 1939), ferret (Gaunt and Hays, 1938) and mouse (Pfeiffer and Hooker, 1940). On the other hand, Gley, et al. (1947) claimed that testosterone propionate given to mature male castrate-adrenalectomised rats would compensate for the loss of the testes, which were held to promote survival. It was further claimed that the administration of 2 mg. testosterone propionate together with 2 mg. DCA to adrenalectomised-castrate rats was more effective than DCA alone and increased considerably the number of animals surviving throughout treatment.

It was difficult to reconcile the contradictory reports of the influence of male and female gonads upon survival, without recourse to hypothetical strain differences. There was no convincing evidence that the testes had any effect upon survival. The report (Gley, et al., 1947) of a significant effect of orchiectomy and of androgen administration was available only in abstract form which provided no detailed results. There was limited evidence that the period for which individual females survived adrenalectomy was related to the presence of the ovary, and to the nature of the reaction of the ovary in the state of adrenal insufficiency. In female hamsters, the occurrence of prolonged dioestrous stages was associated with extended survival, which resulted presumably from the presence of effective levels of progesterone (Snyder and Wyman, 1951). Extended survival in a proportion of adrenalectomised female rats similarly was found in association with prolonged intervals between oestrous periods (Wyman, 1928). Considerable individual variation in the rapidity and nature of the effect of adrenalectomy upon the oestrous cycle of adrenalectomised rats has been recorded (Corey, 1934; Kroc and Martin, 1934; Martin, 1932), but this variation was not contrasted with differences in length of survival.

The apparent strain differences, in the effect of the sex of adrenalectomised rats upon survival, may have reflected the proportions of individuals showing different types and degrees of ovarian reaction. Where the ovaries of the majority of adrenalectomised females reacted in such a manner that effective levels of progesterone, or other corticomimetic compounds, were secreted; a result similar to that obtained by Cowie (1949a) might be expected. On the other hand, where affective levels of progesterone were not produced, or were produced in relatively few animals, and oestrogens were secreted at a level

capable of causing inhibition of survival; a finding such as that of McKeown and Spurrel (1940) could result. The lack of detectable differences between the mean survival periods of male and female rats (e.g. Gaunt, 1933) could result in situations between the above extremes.

A number of hypotheses have been advanced to explain the effects of adrenalectomy upon the ovary (Parkes, 1945). The balance of the evidence suggested pituitary mediation of the effect, although there have been no comprehensive studies of the cytological changes in the pituitary. There has been no convincing report of changes in the levels of pituitary hormones other than adrenocorticotrophin (ACTH), the level of secretion of which is increased considerably after adrenalectomy (Gemzell, van Dyke, Tobias and Evans, 1951; Sydnor and Sayers, 1952). It has been stated (Zuckerman, 1953) that the functions of the gonadotrophic and adrenocorticotrophic hormones overlap. ACTH has been shown to inhibit vaginal cornification and to induce luteinisation in the ovaries of intact mice of the CHI strain (Flux and Munford, unpublished), and to stimulate the production of a corticomimetic compound from the ovaries in adrenalectomised mice (Clayton and Prunty, 1951). It seemed possible that the survival value of the ovary in adrenalectomised virgin females depended upon the secretion of progesterone, which resulted from stimulation by ACTH.

It has been tacitly assumed in the foregoing discussion that the corticomimetic substance causing increased survival in pregnant, pseudopregnant and virgin females was progesterone. While the administration of progesterone has a similar effect to naturally or artificially induced luteinisation of the ovaries, it does not necessarily follow that the survival value of the ovary (or for that matter the placenta) depends upon the production of progesterone. It has recently been shown that the corpus luteum contains two enzymes (6β-hydroxylase

and 21-hydroxylase), which could convert progesterone to the steroid Preg-4-en-6β : 21-diol-3:20-dione (6β-hydroxydesoxycorticosterone). This compound, closely related to DOC has been shown to be an active corticoid (Dorfman, 1954). The presence of cortisol, or a closely related biologically active corticoid, has been demonstrated in placental extracts (Johnson and Haines, 1952) and this could contribute to the prolonged survival observed in pregnant animals.

(d) Diet; Levels of Sodium and Potassium Salts

While a variety of dietary regimen and non-hormonal treatments have been reported to alleviate the effects of adrenal insufficiency, only the specific effects of the ions of sodium and potassium have been substantiated. The administration of sodium salts, orally or parenterally, has a palliative effect in the adrenalectomised animal, which is aided by a reduced intake of potassium (Swingle and Remington, 1944).

Slight alleviation of adrenal insufficiency in dogs and cats, as a result of administration of a variety of solutions of sodium salts, was reported by a number of early investigators (Banting and Gairns, 1926, Corey, 1926, 1927; Soddu, 1898; Zwemer, 1925, 1927). The effect of fluid administration was of doubtful significance; the preponderance of very short survivals suggested that any palliative action was related to post-operative shock rather than adrenal insufficiency.

Later reports indicated that the administration of sodium salts could lead to marked, but not necessarily indefinite, prolongation of the period for which dogs and cats lived after adrenalectomy. Injection of large volumes of Ringer's solution aided survival in adrenalectomised dogs (Rogoff and Stewart, 1928a), as did the addition of substantial amounts of sodium salts to the feed or drinking fluid (Swingle, Pfiffner, Vars and Parkins, 1934; Harrop, Soffer,

Nicholson and Strauss, 1935). Injection of various sodium salt solutions afforded partial protection against adrenal insufficiency in cats (Marine and Baumann, 1927), but did not compensate for the deleterious effects of repeated handling (Hartman, Brownell and Hartman, 1930). Limited alleviation of the effects of adrenal removal resulted from oral administration of sodium salts to adrenalectomised cats (Zwemer, 1934).

Kendall and his associates developed a diet low in potassium and high in sodium salts, which was taken readily by most dogs, and were able to maintain adrenalectomised dogs in good health for an indefinite period by feeding this diet to animals allowed to drink a solution of sodium chloride and sodium citrate (Allers and Kendall, 1937). Cleghorn, et al. (1939) confirmed this finding. Grollman (1952) found that the high-sodium, low-potassium dietary regimen was not uniformly satisfactory and reported a method of peritoneal lavage with a salt solution--ionic composition in m.eg./litre: sodium 150, chloride 107, calcium 40, bicarbonate 25, potassium 4.5, magnesium 1.5--which obviated the use of adrenal cortex extract in the immediate post-operative period.

The first report of prolonged survival in the rat, following the administration of salt solution, was due to Rubin and Krick (1933). This report, however, was not supported by detailed results. Other workers (Gaunt, Tobin and Gaunt, 1934, 1935; Kutz, McKeown and Selye, 1934; Weiser and Norris, 1936) confirmed that administration of the Rubin and Krick solution deferred death indefinitely in a proportion of adrenalectomised rats. There has been no report establishing that the inclusion of cations other than the sodium ion added to the effectiveness of this solution, which was not shown to be superior to normal saline (Gaunt, et al., 1935). Cowie (1949) and Grollman (1941) found that the addition of sodium chloride to the drinking fluid of adrenalectomised rats, of the hooded Norway strain and an albino colony respectively, extended survival in

the majority of animals, but not for an indefinite period. On the other hand there have been reports (Anderson, Joseph and Herring, 1940; Ingle, Ginther and Nezamis, 1943) of complete and indefinite protection of adrenalectomised rats allowed to drink a 1 per cent sodium chloride solution.

The results reported by Ingle, et al. (1943) for adult rats of the Sprague-Dawley strain contrasted with those obtained by Gaunt, et al.(1935) with adults of the Wistar strain. All animals of the Sprague-Dawley strain lived through the period during which access was allowed to saline, but all died when water was substituted for the saline drinking fluid. Approximately one quarter of adrenalectomised rats of Wistar origin did not respond to added sodium chloride while two-thirds of those responding did not die, when the saline drinking solution was replaced by water. The observations of Gaunt, et al. (1935), on immature rats of Wistar origin, differed from those of Cowie (1949b), on young rats of the hooded Norway strain. Approximately 50 per cent of the immature Wistar rats responded to saline, which in these animals afforded complete protection, while the remaining 50 per cent were not affected by the addition of sodium chloride to the drinking water. All immature rats of the hooded Norway strain showed a degree of response, but saline did not afford complete protection to any animal.

In the case of the adrenalectomised mouse the addition of sodium salts to the drinking water was reported by Howard (1946) to promote extended survival in approximately a third of sixty-seven immature mice of the H strain. The provision of a diet with a high sodium and low potassium content was more effective--twelve of fourteen adrenalectomised mice survived for more than three weeks--by this investigator. On the other hand, Hill (1948) could not detect any effect of the addition of either 0.5 or 1 per cent of sodium chloride to the

drinking water in adrenalectomised mice of the CHI and Swiss albino strains. Marder (1950) claimed that the provision of saline drinking fluid and a bread and milk diet promoted indefinite survival on mice of the CBA strain, but was comparatively ineffective in mice of the A and C57 (black) strains. Pfeiffer and Hooker (1940) had earlier failed to obtain extended survival in adrenalectomised mice of the A strain provided with a 2 per cent sodium chloride drinking solution. The contrasted response of CBA mice and of mice of the CHI and A strains was the more remarkable because these strains were descended from common ancestors (Strong, 1942). However, the results obtained by Marder (1950) must be viewed with caution; this worker did not employ a satisfactory test of complete adrenal ablation (Cf. ante, pp. 6 et seq.).

Some observers have noted that feeding bread to adrenalectomised rats (Cleghorn, et al., 1936; Swann, 1937) prolonged the period of survival. Cleghorn, et al. (1936) found that whereas the efficacy of different breads varied approximately according to sodium content, bread having a given content of sodium chloride was more effective than normal diets having the same sodium content. It was suggested that this effect was in part due to the readiness with which rats ate bread and so reflected the total sodium intake. The low potassium content of bread was probably in part responsible for this effect (Zwemer and Truskowski, 1937).

There appeared to be considerable variation, between different strains of rats and mice, in the effect of an increased intake of sodium salts upon survival. In certain strains of rat, the response of individual animals from the same colony was subject to wide variation.

While differences in the potassium content of the diets employed may have been a cause of variation between the results of different workers, the contrasting responses of individual rats (Gaunt, Tobin and Gaunt, 1935), and the conflicting

effects of the same dietary regimen in three strains of mice (Marder, 1950) indicated a source of variation other than the levels of sodium and potassium in the diet. Gaunt, et al. (1935) suggested that individual differences in adrenalectomised rats of the same colony were related to the degree with which adrenal rests assumed functional significance. Variations in the degree to which appetite for sodium chloride was increased by adrenalectomy (Richter, 1936; Richter, Rogers and Mall, 1950); in the extent to which intestinal absorption of sodium ions was reduced after adrenalectomy (Clark, 1939) were further possible causes of species, strain and individual differences in the effect of sodium chloride administration.

(e) Replacement Therapy

(i) Adrenal Extracts. A considerable number of maintenance experiments, in which the endogenous adrenal cortex secretion was replaced by a variety of cortical extracts, were carried out prior to the isolation, characterisation, and synthesis (in quantity) of specific adrenal steroids. The first active adrenal extracts, which served as an adequate substitution therapy for an indefinite period in adrenalectomised animals, were prepared by Swingle and Pfiffner (1930) and Hartman and Brownell (1930).

A variety of extracts obtained from different sources by diverse procedures have been employed and over a period of years these extracts have increased in purity and potency. It was, therefore, difficult to compare the results obtained in different investigations save on the basis of weight of fresh adrenal tissue used as a starting material, or in terms of activity relative to a reference material such as desoxycorticosterone (DOC) or its acetate (DCA). Noble (1950) employed both these methods of comparison in summarising the relative cortin requirements of adrenalectomised animals of a number of species. The adult dog and guinea-pig required approximately the same amount of cortin per unit of body

weight to prevent the appearance of the symptoms of adrenal insufficiency. The cat required about ten times as much cortin per kilogram of body weight as the dog. On a similar body weight basis, the immature rat--to maintain a normal growth rate--required 300 times that amount of extract which would maintain normal blood values in the adult dog.

(ii) Adrenal Steroids: DOC and Related Steroids.

Steiger and Reichstein (1937) described the partial synthesis of crystalline desoxycorticosterone and noted the activity of DCA in adrenalectomised rats and dogs. This observation has been confirmed and extended to a number of esters of DOC by later reports.

At levels as low as 20 ug. daily DCA protected a substantial proportion of immature adrenalectomised rats from the fatal consequences of adrenal insufficiency (Kuizenga, Nelson and Cartland, 1940; Leathem and Wolf, 1954), but the lowest reported level of DCA which would maintain all treated adrenalectomised rats, and promote a normal growth rate for a period of twenty days was 100 ug. per day (Olson, Jacobs, Richert, Thayer, Kopp and Wade, 1944). Other workers have found, however, that ten times this level was required to provide for normal growth (Grollman, 1939, 1941; Masson, Corcoran and Page, 1950).

In the adrenalectomised dog the daily requirement of DOC or DCA for the maintenance of body weight and normal blood composition has been found to lie in the range 10 to 50 ug. per kilogram of body weight (Cleghorn, Fowler, Wenzel and Clarke, 1941; Mason, 1939; Olson, et al., 1944; Remington, Parkins, Swingle and Drill, 1941; Swingle, Maxwell, Ben, Baker, LeBrie and Eisler, 1954a). The level of DCA necessary to maintain adult cats and guinea pigs after adrenalectomy was reported to be of the order of 600 to 1000 ug. per day (Clark, 1941; Hartman, Lewis, Gabriel, Spoor and Brownell, 1940) while 0.25 mg.

daily sufficed to protect mature adrenalectomised mice (Pfeiffer and Hooker, 1940).

The level of DCA required in the adrenalectomised dog was markedly increased when the dietary level of sodium was reduced (Cleghorn, et al., 1941). Grollman (1941) reported a similar observation in the immature adrenalectomised rat, but Eversole (1945a) found that DCA was still partially effective at a level of 200 ug. daily in adrenalectomised rats fed a diet almost devoid of sodium salts; a reduced level of carbohydrate (Eversole, 1945b) had a more profound effect than reduced intake of sodium chloride. The provision of 1 per cent saline produced toxic reactions to DCA administration, at a level which would maintain body weight in adrenalectomised mice drinking water (Pfeiffer and Hooker, 1940).

Differences in the activities of DOC, DCA and a variety of other esters have been assessed using the effect of single injections of these compounds upon the life-span of adrenalectomised animals. The activity of the various esters investigated was qualitatively similar to that of DOC, but in most cases the effect of the esterified steroids was of greater duration (Gaunt, Leathem, Howell and Antonchak, 1952; Gross and Tschopp, 1952; Miescher, Fischer and Tschopp, 1938; Swingle, Collins, Barlow and Fedor, 1952a). The trimethylacetate had the longest action of the compounds tested by subcutaneous injection in rats (Gaunt, et al.,1952) or by intramuscular injection in dogs (Swingle, Collins, et al., 1952a). The phenylacetate also markedly prolonged the period of good health in adrenalectomised dogs when given intramuscularly as a microcrystalline suspspenion (Swingle, Collins, et al. 1952a). In adrenalectomised rats this ester was slightly less effective than the trimethylacetate when given as a crystalline suspension, but was only slightly more effective than either DOC or DCA when given in oil (Gaunt, et al., 1952). When administered subcutaneously to adrenalectomised rats, the duration of activity of the benzoate,

palmitate and butyrate was approximately twice that of the free alcohol, acetate and propionate (Miescher, et al., 1938).

The water-soluble desoxycorticosterone glucoside maintained adrenalectomised dogs and immature rats when given by intramuscular and subcutaneous injection, but the effective dosage was approximately six times that for DCA (Cosmos, Duell and Gaunt, 1950; Swingle, Perlmutt, Seay and Collins, 1952). Conversely, the glucoside was much more effective than the acetate when administered intravenously to dogs in the terminal stages of adrenal insufficiency (Swingle, Perlmutt, Collins, Seay, Fedor and Barlow, 1951; Swingle, Perlmutt, et al., 1952).

The differing effectiveness of the various derivatives of DOC appeared to be related to their solubilities and thus presumably reflected the rate of absorption from the injection site. However, Gaunt, et al., (1952) found that the activity of the trimethylacetate, which had a marked effect upon the maximum body weight reached by adrenalectomised animals as well as upon the period of survival, seemed also to depend upon a reduced rate of destruction.

The life-maintaining activities of two steroids related to DOC have been investigated in the adrenalectomised immature rat. 17α-hydroxy-11-desoxycorticosterone (Reichstein's Substance S) (Ingle, 1941; Masson, Corcoran and Page, 1950) and 1-dehydro-11-desoxycorticosterone (Leathem, 1950) promoted survival, but the respective minimal daily requirements were fifteen and nine times that of DCA. However, when the basis of comparison was the effectiveness of a single injection of steroid, Substance S appeared to be equally as potent as either DOC or DCA (Gaunt, Leathem, Howell, and Antonchak, 1952).

(iii) 11-Oxygenated Adrenal Steroids.

The life-maintaining activities of the 11-oxygenated adrenal steroids isolated from extracts of the adrenal cortex have been found to be of a lower order than that of DOC in animals not subjected to stress (Noble, 1950). The

activities of these steroids have been investigated in the adrenal insufficient dog and rat.

The results of early investigations, in which the maintainence of the adrenalectomised dog--the absence of symptoms of adrenal insufficiency; in particular the absence of abnormal blood levels of urea and sodium and potassium ions--was the criterion of activity (de Fremery, Laqueur, Reichstein, Spanhoff and Uyldert, 1937; Kendall, 1937, 1941; Mason, 1939; Mason, Hoehn and Kendall, 1938) have been reviewed by Noble (1950) and by Pfiffner (1942). Corticosterone, the most potent of the 11-oxygenated-compounds, was about one-seventh as active as DCA. Dehydrocorticosterone was slightly less active than corticosterone while cortisol and cortisone were the least potent having less than one-twentieth the activity of DCA. The amounts of the free alcohols of cortisone and cortisol required in the adrenalectomised dog were least when given as an acqueous solution containing 10 per cent of alcohol; the activity relative to DOC of both these steroids was between one-twentieth and one-fortieth (Swingle, Baker, Eisler, LeBrie and Bannick 1955c).

The comparison of DOC and 11-oxygenated corticoids in the immature adrenalectomised rat--with survival and growth rate the joint criteria of activity (Cartland and Kuizenga, 1936)--was complicated by the action of 11-oxygenated steroids upon body weight. However, although corticosterone or cortisone caused a loss per se in the body weight of both normal and adrenalectomised animals while alleviating adrenal insufficiency on other respects (Ingle, 1940, 1941), this was held not to interfere with the evaluation of life-maintaining activity (Kuizenga, Nelson and Ingle, 1943) in the adrenalectomised immature rat.

On the basis of ability to maintain the immature adrenalectomised rat, Kuizenga and his colleagues reported that DCA was six and nine times as active as corticosterone and dehydrocorticosterone respectively (Kuizenga and Cartland,

1939; Kuizenga, Nelson and Cartland, 1940). Similar relative potencies for these two steroids were found by Simpson and Tait (1952) using DOC as a reference standard. Cortisol and cortisone were reported by Kuizenga and Cartland (1939) to be one half and one quarter as potent as corticosterone respectively, but in a later study (Kuizenga, et al., 1943) the activity of cortisone appeared to be eight times greater--approximately one third that of DCA. The results obtained with cortisol and cortisone in the more comprehensive investigation by Simpson and Tait (1952) were in agreement with the earlier (Kuizenga and Cartland, 1939) finding. The apparently conflicting results obtained with cortisone under the same conditions by Kuizenga and his colleagues probably merely indicate the need for larger numbers of animals and adequate statistical procedures in the determination of relative potencies.

Despite the assertion of Kuizenga, et al. (1943) (Cf. ante), it seemed that the activity of cortisol or cortisone was relatively greater when the estimation was based solely upon survival, than when it was determined by a survival-growth method. Thus cortisol and cortisone (Tolksdorf, Battin, Cassidy, MacLeod, Warren and Perman, 1956) or cortisone acetate (Leathem and Wolf, 1954; Wolf, 1955) were approximately half as effective as DCA in promoting the survival of young adrenalectomised rats.

(iv) Aldosterone.

The existence of a highly active compound, such as aldosterone, could have been predicted from pre-war studies (Kuizenga and Cartland, 1939; Mason, 1939; Wintersteiner and Pfiffner, 1936). These demonstrated that the greater part of the biological activity effective in prolonging the life of the adrenalectomised animal, under low stress conditions, was associated with the amorphous residue remaining after the crystallisation of the known hormones. The evidence that this activity was due to an adrenal hormone was recently provided by the isolation

of aldosterone from adrenal extracts, (Simpson, Tait, Wettstein, Neher, v. Euw and Reichstein, 1953; Mattox, Mason and Albert, 1953) and from the adrenal effluent of a number of species (Simpson and Tait, 1955a,b).

The potency of aldosterone in prolonging the life-span of adrenalectomised animals has been evaluated in the adrenalectomised dog. Gross and Gysel (1954) found that aldosterone was 25 to 30 times as active as DCA while Swingle, Maxwell, Ben, Baker, LeBrie and Eisler (1954a,b) reported a potency between 12 and 25 times that of DOC and approximately 500 times cortisol.

(v) Adrenal Steroids with Halogen Substituents or Additional Double Bonds.

The discovery of the surprising bioactivity of 9 alpha bromocortisol acetate (Fried and Sabo, 1953) stimulated the examination of a variety of steroids, with halogen substituents at carbon 9, for corticoid activity.

Fried and his colleagues (Borman, Singer and Numerof, 1954; Fried, Herz, Sabo, Singer and Numerof, 1955; Fried, Thoma, Perlam, Herz and Borman, 1955) utilised the effect of single injections of steroid upon the life-span and growth rate of the immature adrenalectomised rat (Gaunt, et al., 1952) to assess the activity of certain halo-derivatives of cortisol, cortisone, corticosterone and dehydrocorticosterone. The activities of these compounds (relative to DCA = 1) were: fluorocorticosterone, fluoro-B-acetate, fluorodehydrocorticosterone and fluoro-A-acetate, 20-26; chloro-E-acetate and chloro-F-acetate, 10-20; bromo-E-acetate, 5-10; fluoro-E-acetate, 3-5; bromo-F-acetate, 2-5; fluoro-F-acetate, less than one. The extent to which this considerably enhanced activity of the 11-oxygenated adrenal steroids, when a halogen substituent was present at carbon 9, was a reflection of slower absorption from the injection site, or of a lower rate of destruction of corticoid activity in the body was problematical. Leathem and Wolf (1954) reported that chloro-F-acetate was less than twice as effective as DCA when the basis of comparison was the minimal daily level of each steroid

required to ensure the survival of 100 per cent of young adrenalectomised rats. The lower relative potency of chloro-F-acetate obtained by Leathem and Wolf (1954) suggested that, at least in the case of this steroid, the potency reported by Fried and his colleagues represented more efficient action by the halogenated derivative rather than inherently enhanced activity.

Swingle, Baker, Eisler, LeBrie and Brannick (1955a,b) have estimated the life-maintaining activities of chloro-F-acetate and fluoro-derivatives of corticosterone, cortisol, 11β-hydroxyprogesterone and 11β,17α-dihydroxy-progesterone using the daily maintenance requirement of the adrenalectomised dog as the basis of comparison. The potency of fluorocortisone and fluoro-B-acetate (relative to DOC=1) in the adrenalectomised dog was similar to that found in the rat (Fried, Herz, et al., 1955). These derivatives of corticosterone had an activity equivalent to that of aldosterone (Swingle, et al., 1955a). In the dog fluoro-F-acetate was three times more effective than chloro-F-acetate. This contrasted with the result obtained by Borman, et al., (1954) with the single injection method in the rat, but the estimates of the potency of chloro-F-acetate relative to DCA or DOC--on the basis of daily maintainence requirements--were of similar order in the dog (Swingle, et al., 1955b) and rat (Leathem and Wolf, 1954).

The differences between the results of Swingle and his colleagues, on the one hand, and Fried and his co-workers, on the other, probably reflected a difference in the methods used to evaluate activity rather than a species-difference in activity. In this connection it was of interest to note that fluoro-F-acetate was twice as active as chloro-F-acetate in causing sodium retention in the adrenalectomised rat (Fried, Thoma, et al., 1955).

The activity of the fluoro-derivative of 11β-hydroxprogesterone was of the same order as that of DCA in the adrenalectomised dog, while 9α-fluoro-11β: 17α

-dihydroxyprogesterone was slightly less active than DOC (Swingle, et al., 1955b). These two steroids could be regarded as derivatives of corticosterone and cortisol--obtained by reduction of the hydroxyl function at carbon 21--and the lower activity of the cortisol "derivative" (the dihydroxy- compound) was consistent with lower activity of cortisol, when compared with corticsterone.

The introduction of a further double bond, between carbons 1 and 2, into the nucleus of the molecules of cortisol and cortisone produced a three fold increase in life-maintaining activity (Tolksdorf, et al., 1956); the daily requirement of 1-dehydrocortisol or 1-dehydro-cortisone, for the protection of the adrenalectomised rat was slightly less than that of DCA.

The synthesis of steroids containing a halogen substituent, together with an additional double bond in the nucleus, has been reported by Fried, Florey, Sabo, Herz, Restivo, Borman and Singer, (1955). Preliminary investigations of the sodium-retaining abilities of two of these steroids, 1-dehydro-9alpha-chlorocortisol and 6-dehydro-9-alpha-fluorocortisone, indicated an activity in this respect of the same order as that of aldosterone.

B. THE ROLE OF THE ADRENAL CORTEX IN THE DEVELOPMENT OF THE MAMMARY GLAND

Evidence of the part played by the adrenal cortex in the endocrine control of mammary growth has been provided by investigations of the effect of adrenalectomy; by studies of the effects--in intact animals, and in animals lacking one or more endocrine glands--of the administration of extracts of the adrenal cortex, or of specific adrenal steroids. Further indications of a possible role of the adrenal cortex have come from studies in which steroids, known to have mammogenic activity, have been isolated from adrenal extracts, and from examinations of the effect of the adrenal cortices upon the mammary gland, in abnormal physiological conditions.

1. The Effect of Adrenalectomy

Adrenal removal has been reported to promote regression of existing mammary gland structure and to reduce the effect of mommogenic stimuli (Cramer and Horning, 1939; Smith, 1951; Trentin and Turner, 1947, 1948b); to stimulate mammary development (Butcher, 1939; Johnston and Smithcors, 1948; Reeder and Leonard, 1944); or to be without consistent effect on the mammary gland (Chamorro, 1946a; Cowie, 1949b; Cowie and Folley, 1947; Jacobsohn, 1949).

Adrenalectomy has been claimed to cause regression of the mammae of mature mice (Cramer and Horning, 1939) and to intensify the atrophic changes which follow castration in the mature male rat, of the Wistar strain, (Trentin and Turner, 1947, 1948b). The response of the mammary gland to oestrogen administration appeared to be reduced in both species following adrenal removal. The conclusions reached by Cramer and Horning and by Trentin and Turner would have been more convincing had they been based on a greater number of animals,

and had more than one gland from each animal been used to assess treatment differences. The evidence provided by Cramer and Horning was rendered less convincing by the unknown influence of functional accessory cortices, the presence of which was suggested strongly by the extended survival shown by the majority of their adrenalectomised mice. Accessory cortical tissue, from the results of a preliminary survival trial, was assumed by Trentin and Turner (1947) to be functionally unimportant in the colony of rats from which they obtained their experimental animals. In a brief abstract, Smith (1951) reported that adrenalectomy reduced the response of the mammae of ovariectomised, immature rats to treatment with oestrogen and progesterone. The provision of drinking fluids containing 1 per cent of sodium chloride or glucose did not affect this inhibition.

In a brief report, unsupported by detailed results, Butcher (1939) claimed that, in underfed female albino rats, the mammary glands grew faster in the absence of the adrenal glands. The adrenalectomised rats were apparently maintained during the experiment by the provision of access to a 3 per cent solution of sodium chloride. Reeder and Leonard (1944) reported that adrenalectomy stimulated increased branching of the duct system of the mammary glands of intact, and oestradiol-treated intact and castrate immature male mice of the Long-Evans and Sprague-Dawley strains. Johnston and Smithcors (1948) obtained augmented stilboestrol-induced duct growth in castrate albino rats in the absence of the adrenals. The conclusions of both these groups of workers depended upon the subjective appraisal of a single mammary gland from each rat. The

strain * of animals used by Reeder and Leonard (1944) were known to possess accessory cortices, but no results of any tests of the completeness of adrenal removal were reported. Further, reference was made by these authors to the strong positive relationship between body weight gain and degree of duct branching in the mammary gland, within the several groups of adrenalectomised animals. It seemed that the greatest effect of "adrenalectomy" was obtained in those animals in which adrenal rests--subjected to acute stimulation by ACTH (Gemzell, et al., 1951; Sydnor and Sayers, 1952)--were present. Johnston and Smithcors (1948) carried out histological examinations for accessory cortices at autopsy, but did not report either the incidence of accessories, or any differences due to their presence. In view of the undoubted (Reeder and Leonard) or possible (Johnston and Smithcors) presence, but quantitatively unknown influence of adrenal rests, it was difficult to assess the value of the conclusions reached by both these groups of authors.

In a series of experiments involving very small numbers of virgin female rats, Chamorro (1946a) did not demonstrate any effect of adrenalectomy upon established structure, but claimed that adrenalectomy reduced the effect of some mammogenic stimuli (thyroidectomy) and enhanced that of other stimuli (administration of a pituitary preparation in the presence of the ovaries). The very limited animal numbers, and the restricted subjective assessment of the changes in the mammary glands, made the importance of these findings difficult to determine. Cowie and Folley (1947) reported a detailed investigation of

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* The authors did not indicate which of the two strains used was known to possess accessory cortical tissue. It appeared unlikely that this was the case with rats of the Sprague-Dawley strain since a number of other workers have obtained mortality rates of 100 per cent after adrenalectomy in animals of this strain (e.g. Ingle, et al., 1943)

the effects of adrenalectomy upon the mammary glands of gonadectomised hooded Norway rats of both sexes. There was slight evidence that adrenalectomy depressed the response of the mammae to anterior pituitary extract, but no suggestion that either the adrenals (or the gonads) were essential for its action. In two out of three series of animals slight regressive changes on established structure appeared to result from adrenalectomy, but there was no striking evidence of a specific role of the adrenals in the maintainance of mammary structure. Cowie and Folley based their conclusion upon measurements of the area covered by the duct system of all glands in each rat, and upon independent semi-quantitative grades assigned to degree of arborescence of the duct systems, and extent of alveolar development. In a further study, Cowie (1949b) found that adrenalectomy did not affect the relative growth rate of the mammary glands--increase in the extent of the duct system in relation to the age-increase in an index of general body surface--in the female hooded Norway rat. However, the mammae of adrenalectomised male rats appeared to have a significantly greater ($p<0.01$) relative growth rate than those of intact male rats. Cowie doubted the biological significance of this latter finding and suggested that it arose from the preponderance, in the older age groups, of animals possessing functional but histologically undemonstrated adrenal rests.

Although the reported effects of adrenalectomy upon the mammary gland appeared contradictory, the majority of the results obtained have a common quality: the failure to demonstrate a consistent and remarkable effect of adrenal removal upon the mammae. The inconclusive effect of adrenalectomy would appear to preclude the assumption that the adrenal glands have a specific role in either the development or maintenance of mammary gland structure. In view of the severe effects of adrenalectomy upon the animal as a whole (Noble, 1950, 1955) and in view of the findings reviewed above the adrenals appeared to

be involved in normal mammary gland development, but their action seemed to be "permissive" rather than specific.

This conclusion was borne out by the results obtained by Jacobsohn (1949) who reported that adrenalectomised rats, maintained in normal physiological condition by parabiotic union with adrenal-intact rats, showed a similar degree of mammary development as intact rats in response to mammogenic stimulation. The normalising effect of the adrenal-intact parabiont upon the adrenalectomised co-twin did not appear to depend upon the passage of adrenal steroids (Jacobsohn, 1949); a finding in agreement with other evidence (Finerty, 1952) that steroid hormones did not pass the "parabiotic barrier".

2. The Effect of Increased Levels of Adrenal Steroids

The administration of adrenal steroids and the stimulation of the endogenous cortical secretion, by treatment with ACTH, have been employed to determine the effect of increased levels of corticosteroids upon the mammary gland.

(a) Stimulation of the Endogenous Adrenocortical Secretion

ACTH has been reported to stimulate the growth of the mammary glands of intact (Johnson and Meites, 1955; Nelson, 1941), gonadectomised, hypophysectomised, and gonadectomised-hypophysectomised (Nelson, 1941) rats; it has been claimed to enhance oestradiol-induced development and to increase secretory activity of the mammae in ovariectomised rats (Selye, 1954b). On the other hand, ACTH did not affect the mammae of ovariectomised mice of the CHI strain, but inhibited oestrone-induced growth in area and arboresence in ovariectomised mice (Flux, 1954). In intact mature virgin female mice of the CHI strain ACTH was without significant effect upon the mammary glands (Flux and Munford, unpublished).

Comparison of the results reported with the two species suggested that the reaction of the mammary glands of mice of the CHI strain to exogenous ACTH was

very different from that reported with rats. The suggested differences, however, were not conclusive and required confirmation over a greater range of ACTH levels (in the rat) and in other strains of mice.

The apparent effect of ACTH in the rat was considered (Selye, 1954b; Johnson and Meites, 1955) indicative of a specific role of the endogenous "glucocorticoid" secretion in mammary development. This conclusion would have been more convincing had objective measures of different features of the mammae been employed in place of the limited subjective assessment of overall effects of ACTH on the mammae. Flux (1954) has indicated the need to consider growth in extent and increased duct branching independently in assessing the response of the mammae. This worker found that an increase in the density of duct junctions could accompany marked inhibition of the extent of the gland and of the total number of duct junctions in the mammae. Examined in histological sections (e.g. Selye, 1954b; Johnson and Meites, 1955) such increased density of duct branching might well be mistakenly interpreted as indicating specific stimulation of lateral duct development.

In view of the effect of ACTH upon the ovaries (Clayton and Prunty, 1951; Flux and Munford, unpublished, cited in a previous section of this review p.30) the conclusion that the effect of ACTH in the intact rat was mediated entirely by the adrenal cortex (Johnson and Meites, 1955) seemed unjustified. The quantity of crude material (10 - 25 mg./day) injected by Nelson (1941) was so great that the possible effects of contaminants in the ACTH preparation could not be disregarded (Flux, 1954). The lack of effect of the preparation in adrenalectomised animals, in view of the very considerable variation in response in adrenal-intact animals, could have resulted from the general physiological inadequacies of the adrenalectomised animal; it did not, in the absence of detailed results, provide conclusive evidence of adrenal mediation.

While the effects of ACTH on the mammae observed by Selye and by Johnson and Meites appeared to be similar, the level of ACTH employed by the former worker was more than 10 times that used by the latter workers. This apparent difference in sensitivity emphasized the need for an examination of the effects of several levels of ACTH in the rat.

(b) Administration of 11-Oxygenated Adrenal Steroids

Selye (1954a,b) claimed that cortisol (0.5 mg./day) caused "slight mammary development, characterised mainly by duct proliferation", in intact, adrenalectomised, and adrenalectomised-ovariectomised female rats of the Sprague-Dawley strain; oestradiol-induced mammary development was enhanced and secretory activity stimulated in ovariectomised and adrenalectomised-ovariectomised rats. A qualitatively similar effect was reported by Johnson and Meites (1955) who claimed that cortisol or cortisone (2 mg./day) induced duct branching, alveolar development, and secretory activity in the mammae of intact female rats of Wistar origin.

Results which contrasted markedly with those obtained with the rat were reported by Flux (1954) for CHI mice. Cortisone acetate (50 and 100 ug./day) inhibited the growth of the mammae of intact and oestrone-treated ovariectomised mice, both the growth in area and increase in the number of duct junctions of the glands being affected. Cortisol (100 and 200 ug./day) and 11-dehydrocorticosterone acetate (200 ug.), had qualitatively similar significant effects upon oestrone-induced mammary development, while corticosterone had a similar but non-significant effect at the single dose level (100 ug./day) used. No effect of cortisone acetate could be demonstrated upon the mammary glands of immature ovariectomised mice. not treated with oestrone.

In each of the rat and mouse, the effect of 11-oxygenated adrenal steroids on the mammae was qualitatively similar to that of ACTH. The reactions of the

mammary glands in these species appeared to be diametrically opposite, but any conclusion regarding a species difference was subject to considerable reservation. Selye (1954b) and Johnson and Meites (1955) have chosen to interpret the effects of cortisol and cortisone in the rat as indicative of a specific role of endogenous "glucocorticoids" in normal mammary development. This conclusion lacked conviction and the reservations regarding the results on which it was based were similar to those stated in relation to the effect of ACTH (see previous page.).

Selye (1954a) appeared to have been the only investigator to employ adrenalectomised animals in the investigation of the effects of the 11-oxygenated steroids. Information regarding the effects of adrenalectomy in the strain of rats used was not provided. However Ingle, et al. (1943) had previously shown that rats of this strain (Sprague-Dawley) were devoid of functional adrenal rests and were uniformly protected from the effects of adrenalectomy by the addition of 1 per cent of sodium chloride to the drinking solution. Selye claimed that the most striking effect of cortisol administration was obtained in adrenalectomised-ovariectomised rats receiving 5 ug. oestradiol daily. The rats in question were provided with saline drinking fluid, but it seemed unlikely that this would have sufficed to maintain normal physiological condition in the face of administration of high levels of oestrogen. The level of oestradiol was more than 10 times that amount found by Silver (1953) to give maximal relative growth of the mammae in ovariectomised hooded Norway rats. The effect of cortisol may have been to offset the deleterious effect of oestrogen administration so permitting the full response of the mammae of the adrenalectomised animal to oestrogen stimulation. Evidence suggesting that oestrogen administration adversely affected adrenalectomised rats and increased the cortin requirement for maintenance of life in the adrenalectomised ferret has been

cited in Section A of this review (p.18).

(c) Administration of 11-Desoxycorticosteroids

Desoxycorticosterone (DOC). DOC has been isolated from adrenal extracts (e.g. Steiger and Reichstein, 1937). Although it may be present in trace amounts, this steroid was not considered an important component of the normal adrenal secretion (Bush, 1953; Hechter and Pincus, 1954). Since it did not appear that the results of investigations of the effects of DOC or its acetate (DCA) contributed significantly to the understanding of the role of the adrenal cortex in mammary development, evidence pertaining to the influence of these compounds upon the mammary gland has been cursorily reviewed.

DCA was shown to stimulate marked duct proliferation in intact and castrate male mice of one strain (Van Heuverswyn, Folley and Gardner, 1939); to be without remarkable effect on the mammae of similar mice of two other strains (Chamorro, 1945a; Strait and de Ome, 1947), and to induce a limited response in ovariectomised mice of yet another strain (Flux, 1954). When given in conjunction with oestrogen, DCA caused considerable growth of the duct system in ovariectomised mice of the CHI strain (Flux, 1954) and promoted marked "lobule-alveolar" development in male and overiectomised mice of an albino strain (Mixner and Turner, 1942, 1943; Trentin and Turner, 1948b). Flux (1954) has suggested that the effect of DCA in mice depends upon the presence of another hormone such as an oestrogen; that the varied effects of DCA administration in different individuals and strains merely reflected the presence or absence of such a hormone.

On the one hand DCA has been claimed to promote duct growth (Reeder and Leonard, 1942) and on the other to be without consistent effect upon the mammae (Chamorro, 1945b, 1946b) of the male rat. In both the rhesus monkey (Speert,

1940) and the guinea-pig (Nelson, Gaunt and Schweizer, 1943) DOC has been reported to support considerable mammary development.

17-Hydroxydesoxycorticosterone (Reichstein's Substance S). The effect of Substance S upon the mammary gland was investigated by Flux (1954). Although in other respects Substance S has been reported to have effects qualitatively similar but quantitatively much less than DOC (see Section A of this review) Flux found that this steroid resembled cortisone rather than DOC as regards its effect on the mammary gland of CHI mice; that is Substance S acetate reduced the response to oestrone in ovariectomised mice but was without effect on the mammae of ovariectomised mice not given oestrone.

Substance S has been isolated from adrenal extracts, but is probably of little significance in the normal adrenocortical secretion (Hechter and Pincus, 1954).

(d) Secretion of "Mammogenic" Steroids by the Adrenal Cortex

Several steroids--desoxycorticosterone (see earlier section), progesterone (Beall and Reichstein, 1938), and oestrone (Beall, 1939)--which are known to be capable of causing mammary development have been isolated from adrenal extracts. These have not been demonstrated at appreciable levels in the normal adrenal effluent, but in certain circumstances the adrenal cortex secretion may have considerable oestrogenic or progestational activity.

The release of a progestationally active substance by the adrenal was suggested by recently reported studies with human patients (Davis, Test, Navori, Hyrse, Pottinger and Dunkle, 1952, 1953) and with hypophysectomised-ovariectomised rats (Lyons, Li, Johnson and Cole, 1953). Zarrow and Lazo-Wasem (1955) detected small quantities of a progesterone-like compound--active in the biological test of Hooker and Forbes (1941)--in the systemic blood of untreated castrate rats and rabbits, but could not detect any similarly active substance in the blood of adrenalectomised-

castrate rats. Stimulation of the adrenal resulted in a manifold increase in the concentration of this progestin, which in ACTH-treated animals was present in the systemic blood at levels approaching those obtaining during pregnancy in the ewe (Neher and Zarrow, 1954) and the rabbit (Neher and Zarrow, 1953).

Several investigators have reported that animals ovariectomised early in life developed abnormal adrenal cortices, and showed oestrogen-like stimulation of seconday sex organs (Dorfman and Gardner, 1944; Fekete, Woolley and Little, 1941; Flux, 1953; Woolley, Fekete and Little, 1941). The most likely explanation of these effects appeared to be the secretion, by the abnormal adrenal cortices, of oestogenic substances (Flux, 1953).

CONCLUSIONS DRAWN FROM THE SURVEY OF THE LITERATURE.

Factors Affecting Survival After Adrenalectomy

The more important determinants of the reaction of animals to adrenal insufficiency appeared to be: the operative technique; the balance of sodium and potassium ions in the diet; the species, strain, age and sexual condition of the operated animal.

The technique of adrenal removal influenced firstly the degree of post-operative shock and secondly the completeness or incompleteness of adrenal ablation. In general, a wide sodium:potassium ratio in the diet (or the administration of sodium salts) alleviated the fatal symptoms of adrenal insufficiency; in particular, the effectiveness of controlled sodium-ptassium intake was affected by the presence of accessory cortices, the impotance of post-operative shock, and the species, strain and age of the animal operated.

The age, strain and species could be supposed to determine an inherent and characteristic survival period in a totally adrenal insufficient animal. These factors, however, also exerted an influence on mortality rates and survival times by

virtue of effects upon the occurrence and level of activity of accessory tissue, ease of operation and degree of post-operative shock, and the nature of the response to control of the sodium-potassium balance. The effect of age, strain or species appeared to be modified in turn by the sexual condition of the animal. In so far as conflicting reports of the effects of the testes and non-luteinised ovary were a reflection of age and strain differences the converse seemed also to apply.

The Role of the Adrenal Cortex in Mammary Gland Development

The investigations of the effect of adrenalectomy upon the structures of the mammae did not appear to provide convincing evidence of a specific role of the adrenal cortex in mammary gland growth.

The nature of the action of 11-oxygenated steroids upon mammary gland growth was not clear. In the rat these adrenal steroids have been held to stimulate mammary development, but the inadequacies of the criteria employed to evaluate changes in the mammae rendered the hypothesis of a specific stimulating effect of the adrenal secretion less attractive. In the mouse an increased level of adrenal activity appeared to lead to inhibition rather than stimulation of mammary development.

Steroids such as desoxycorticosterone, progesterone and oestrone, capable of causing mammary development, have been isolated from adrenal extracts, but they have not been demonstrated in appreciable quantity in the normal adrenal secretion.

CHAPTER III

FACTORS AFFECTING THE REACTION OF MICE TO BILATERAL ADRENALECTOMY

Relatively few investigations of the factors affecting adrenal insufficiency have been conducted with the mouse; the most comprehensive published accounts being those of Firor and Grollman (1933), Pfeiffer and Hooker (1940), and Marder (1950). The mice available at the beginning of this study were of a strain of unknown ancestry and limited experimental history. Later, limited numbers of mice of three other strains were available, but only one of these--the CBA strain (Strong, 1942)--had previously been used in an investigation of adrenal insufficiency (Marder, 1950).

As information on the reaction of the experimental animal to adrenalectomy was a prerequisite for a study of the effects of adrenal removal on the mammary gland, the investigations described in this chapter were undertaken. The material presented is divided into three sections. In the first, a preliminary experiment with immature albino mice is described. An account of further investigations with this strain forms the second part while in the final section preliminary studies on three additional strains of mice are reported.

A. PRELIMINARY INVESTIGATIONS OF THE RESPONSE OF IMMATURE ALBINO MICE TO ADRENALECTOMY

The object of this preliminary study was to discover whether:

(i) It was possible to keep adrenalectomised albino mice alive, for an extended period, by the administration of sodium chloride;

(ii) The technique of adrenalectomy employed resulted in complete removal of cortical tissue;

(iii) The effect of operative procedure, per se, could be separated from the effects of adrenal insufficiency.

Materials and Methods

Animals

The mice used were immature males and females of an albino strain maintained by Dr. D'S. Flux at Massey Agricultural College, Palmerston North. This colony was established in 1954 from six pairs of mice of unknown ancestry supplied by Mr. W.O. Jarrett from the colony maintained by him at the Palmerston North Public Hospital.

Diet

The diet, fed ad libitum, was a mixture of dried skim milk powder and cereals--equal parts of barley meal, crushed oats, bran, and skim milk powder--supplemented by additional fat soluble vitamins.

Technique of Adrenalectomy

The operative technique was adapted from that employed by Cowie (1949a) with the rat. Considerable initial difficulty was experienced in obtaining complete removal of the right adrenal gland. This was closely connected to the vena cava, and could not be raised any distance towards the incision without rupture either of the adrenal capsule, or of the vena cava.

A number of methods of exposing the operative site were tried. The procedure finally adopted involved the complete removal of a small portion of the dorso-lateral body wall. This obviated the need for retraction of the body wall, which in the immature mouse, was found to be both clumsy and time consuming. There appeared to be no net ill-effects from the complete removal of a portion of body wall, as compared with the use of a simple incision together with retraction. Thus there were no apparent differences in the survival periods of mice adrenalectomised early in the

study (when a simple incision was employed) and those of animals operated in the latter part of the investigation. It seemed that reduction in the time required to complete the operation offset the effects of any additional trauma.

The sequence of events in the removal of the right adrenal is described below.

The mouse was anaesthetised with ether, the operative area clipped and sponged with 70 per cent (by weight) alcohol, and a skin incision made in the mid-dorsal line. After separation of the body wall and integument by blunt dissection, a triangular portion of the body wall: bounded anteriorally by the ribs, dorsally by the longissimus dorsi, and posteroventrally by a prominent branch of the dorsal aorta; was removed.

Further stages of the operation were carried out with the animal lying on its left side, and orientated at right angles to the operator's front. The adrenal was removed by blunt dissection for which two pairs of opthalmic forceps were employed. Use was made of a dissecting microscope giving a magnification of seven diameters.

The kidney was freed from the dorsal body wall, a grip taken between this organ and the adrenal, and the kidney capsule stripped from the anterior pole of that organ. The connection between the adrenal and dorsal body wall was carefully severed, a fresh grip taken below the adrenal with one pair of forceps and the second pair introduced between the first pair and the vena cava. The gland was removed by sliding the upper pair of forceps over the lower pair and tearing the connection between the adrenal and vena cava. Immediately after removal the adrenal was examined to verify the integrity of the capsule. The site of the operation---particularly the torn end of the adrenal vein---was cauterised prior to the removal of the second pair of forceps.

TABLES II, III, IV.

TABLE II

DISTRIBUTION OF ADRENALECTOMISED, IMMATURE ALBINO MICE ACCORDING TO DEGREE OF ADRENAL ABLATION, DRINKING SOLUTION, AND SEX

DEGREE OF ADRENAL REMOVAL / DRINKING SOLUTION / SEX	Saline		Water	
	Female	Male	Female	Male
Gland Capsule Intact	15	17	15	16
Gland Capsule Pierced	4	4	5	3
Macroscopic Adrenal Remnant	-	-	5	4

TABLE III

NUMBERS OF IMMATURE ALBINO MICE DYING FOLLOWING ADRENALECTOMY

DEGREE OF ADRENAL REMOVAL / DRINKING SOLUTION / SEX	Saline		Water	
	Female	Male	Female	Male
Gland Capsule Intact[1]				
Dying within 36 hrs.	5	1	2	3
Dying after 36 hrs.	10	16	13	13
Gland Capsule Pierced				
Dying within 36 hrs.	0	1	1	0
Dying after 36 hrs.	1	1	3	1
Not Dying	3	2	1	2
Macroscopic Adrenal Remnant[2]				
Dying within 36 hrs.	-	-	0	1
Not Dying	-	-	5	3

[1] No animals survived indefinitely; [2] No animals died after 36 hours.

TABLE IV

MEAN SURVIVAL PERIODS OF IMMATURE ALBINO MICE DYING AFTER BILATERAL ADRENALECTOMY

SEX / DRINKING SOLN.	Saline		Water		Both	
	No.	Mean	No.	Mean	No.	Mean
Female	11	4.09	16	4.25	27	4.19
Male	17	3.71	14	3.86	31	3.77
Both	28	3.86	30	4.07	58	3.97
SIGNIFICANCE OF MEAN DIFFERENCES	No significant differences between: means for male and female sexes ($0.3 > p > 0.2$) or means for saline and water ($0.7 > p > 0.6$)					

Survival times are given in days. Animals dying in the first 36 are excluded.

The left adrenal was removed by essentially the same technique as that described above. In the case of this gland, however, a simple incision was employed to enter the body cavity, and the adrenal was exteriorised through this incision after it had been detached from the dorsal body wall and from the kidney.

The skin incision was closed by two or three interrupted sutures.

With practice, it was found that the complete operation could be performed in less than ten minutes, and only exceptionally was the animal anaesthetised for longer than fifteen minutes. Ether appeared satisfactory for use with immature mice of the albino strain (Cf. Nissim, 1953), provided the period of anaesthesia did not exceed twenty to twenty-five minutes.

Post-operative Care

The mice were disturbed as little as possible after the operation, and were housed in a room maintained at temperatures between 65° and 75°F.

Application of Treatments

Weaned at 21 days from birth, the mice were adrenalectomised within two days of weaning and allotted at random to one or two treatment groups. One of these groups received tap water as a drinking solution while the other was given tap water to which one per cent, by weight, of sodium chloride had been added.

Bilateral adrenalectomy was attempted in a total of 89 male and female mice. In nine animals a macroscopic portion of one gland was left at the site of operation. In a further seventeen mice one of the gland capsules appeared to have been pierced during the operation. The remaining 63 mice were considered to have been completely adrenalectomised in so far as the principal adrenal glands were removed intact.

The distribution of mice in each of the three operative categories is shown in Table II.

Body Weights

The body weight of each mouse was recorded immediately after the operation. No other body weights were recorded.

It has been abundantly shown that adrenalectomised animals were not equipped to withstand even minor stress (e.g. Brogi, 1954). Because it was thought that the act of weighing, by placing the animal under stress, might have hastened death from adrenal insufficiency, no determinations of body weight were made in the period between the operation and death.

The dead weights were not recorded since it was impossible to decide to what extent post mortem weight changes, in the variable period between death and the daily inspection, had contributed to the observed dead body weight.

Survival Periods

Deaths were recorded daily, except during the first 48 hours after the operation when inspections were made at more frequent intervals. The period for which any mouse survived was recorded to the nearest whole day. Thus an animal dying in the interval 36 to 60 hours after the operation was recorded as surviving two days while one dying in the interval 84 to 108 hours was regarded as having survived four days.

Autopsy

Those animals which survived indefinitely were slaughtered at approximately 60 days of age and a macroscopic examination made, at the site of the operation, for residual or accessory adrenal cortical tissue.

Results

Mortality Rate

The mortality rate resulting from adrenalectomy is shown in Table III.

Of the thirteen animals recorded as dying within 36 hours of the operation, the majority died within 12 hours, three lived almost 18 hours, and one animal died about the 24th hour. In examining the effects of the various factors on the development of adrenal insufficiency, only those animals that survived longer than 36 hours have been considered. It was hoped that the exclusion of this group of animals divorced the immediate effect of operative shock from the more delayed effect of adrenal insufficiency. This working hypothesis was not at variance with the results obtained with nine animals subjected to partial adrenalectomy. The only mouse, in this group, that died did so within 12 hours of the operation.

All eight animals which survived superficially complete bilateral adrenalectomy were found in the group in which completeness of adrenal removal was suspect. It seemed probable that, in these animals, the apparent puncture of the capsule of one of the adrenals allowed the escape of cortical tissue into the body. There was no evidence that hypertrophy of accessory cortical tissue was concerned in the indefinite survivals recorded in this experiment.

The influence of sex, drinking solution, and the interaction of these upon the proportions of animals falling in each of the three "survival groups" was examined statistically in a series of 2 x 2 contingency tables. None of the several hypotheses--that the differences observed were due to chance--could be rejected with confidence. The probability that the observed differences could have been due to chance was calculated in each table by Fisher's exact method (Goulden, 1952, pp 372 et seq.) and no value less than 0.25 was obtained.

Residual Cortical Tissue at Autopsy

Eight of the nine mice submitted to a "control" operation, in which

gross portions of one or other of the adrenals was left within the body, survived indefinitely and were slaughtered at approximately 60 days of age. Apparent cortical tissue was macroscopically demonstrable in seven of these eight mice in the region previously occupied by the partially removed gland.

Of the seventeen animals in which a small amount of cortical tissue could have escaped into the body, eight survived to autopsy at 60 days from birth. Macroscopic inspection of the operative site in these animals, however, failed to reveal recognisable cortical tissue.

Survival Period

The mean survival periods, and tests of significance of the differences between the means, of the fifty-eight mice considered to have died as a result of the development of adrenal insufficiency are shown in Table IV.

The analysis of variance technique employed to examine these data was that appropriate (Snedecor, 1946, 285 et sqq.) for the case of disproportionate sub-class numbers where the interaction between "treatments" was negligible. The resultant analysis of the survival periods of male and female mice adrenalectomised and receiving added sodium chloride or normal tap water is presented in Table XVI.

From the results of the analysis of the means presented in Table IV it was apparent that the administration of sodium chloride had not increased the survival period of adrenalectomised mice. Although female mice appeared to survive slightly longer on average than males, the small mean difference was not significant.

The frequency distributions for each of the treatment groups (male, female and saline water) are shown in Fig. 1. The distributions for the two groups of animals receiving the different amounts of sodium chloride appear visually to be identical in form. On the other hand the distributions for the two sexes appear to differ quite markedly in form.

Discussion

Influence of Operative Shock

It was apparent from the proportion of survivals of less than one day, that traumatic and other types of shock implicit in the operative procedure were important. No reports were available regarding the effect of operative shock in mice of comparable age to those used in the present experiment. However, the results reported above did compare with those previously reported (Firor and Grollman, 1933; Sisson and March, 1935; Gillman and Golberg, 1942) for the weanling rat.

In the rat, Gillman and Golberg (1942), on the basis of evidence similar to that considered above (p.44), concluded that the effects of adrenal insufficiency could be freed from the immediate influence of operative shock by excluding those animals which failed to survive 48 hours. This decision, like that made by the present investigator, was largely intuitive. Both decisions however, appeared to draw the dividing line at the most easily distinguished discontinuity in the distribution of deaths in relation to time from bilateral adrenalectomy, and both were supported by limited evidence from control operations. These decisions, which were to be regarded as "working hypotheses", differed only in respect

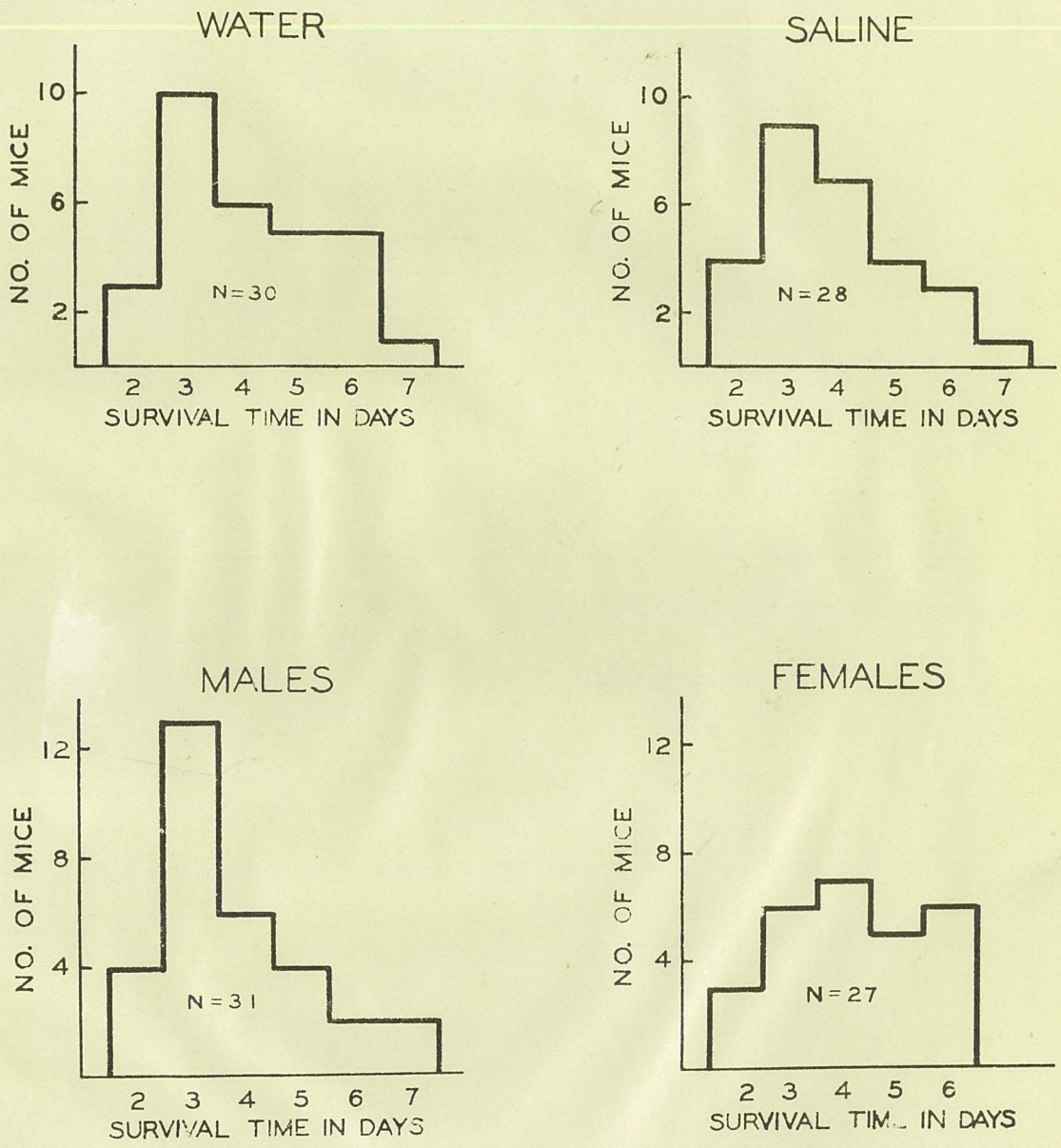

FIGURE 1. FREQUENCY DISTRIBUTION OF THE LIFESPAN OF IMMATURE ADRENALECTOMISED ALBINO MICE.

of the point of demarcation on the physical time scale.

Indefinite Survivals

The results obtained in this experiment did not indicate the presence, or the absence, of accessory cortical tissue. It was concluded that, if accessory cortical tissue was present in the strain of mouse used, either this was removed (or destroyed) at adrenalectomy, or it did not hypertrophy sufficiently to allow indefinite survival.

In the rat it was shown by Gaunt, Tobin and Gaunt (1935) that immature animals exhibited a lesser mortality rate, due to hypertrophy of accessory cortices, after a period of maintenance with saline drinking fluid. In the present study the provision of sodium chloride in the drinking water did not increase survival times. It was not, therefore, surprising that mice receiving saline did not show a decreased mortality rate.

The reduced mortality in the group where completeness of adrenal removal was suspect indicated that quite small amounts of cortical tissue resulted, at least in a proportion of cases, in indefinite survival. In the case of those animals in this group which failed to survive, it was concluded that, either cortical tissue had not escaped into the body through the apparent puncture of the capsule, or such tissue had not become implanted so as to function at a sufficient level to produce indefinite survival.

In this study it was not possible to demonstrate, macroscopically, residual cortical tissue in animals where the presence of this was indicated by indefinite survival. Cramer and Horning (1939) likewise failed to detect residual or accessory cortices in the majority of indefinite survivors. Even when a macroscopic search was combined with careful histological examination of all suspicious tissues in the anterior portion of the abdominal cavity, the presence

of accessory or residual cortical tissue, in indefinite survivors, has not invariably been demonstrated (Cowie, 1949a; Dorfman, et al., 1946; Gaunt, Gaunt and Tobin, 1935).

Survival Times

The results obtained in this investigation suggested strongly that the administration of sodium chloride did not lead to prolonged survival in the immature albino mouse. On the other hand, although the mean difference between male and female survival periods was small and not statistically significant, the frequency distributions for the sexes (Fig. 1) appeared to differ. It was considered that failure to demonstrate a significant difference resulted from a lack of statistical control of intra-sex variation, rather than form the lack of a real difference between the male and female. This supposition has been borne out but the results of later experiments, in which time changes were in part controlled by the segregation of the variance due to differences between block means.

Summary

It was concluded that the major and immediate effect of operative shock could be separated from that of adrenal insufficiency by excluding, in consideration of the latter, those animals dying within 36 hours of adrenalectomy.

Of the animals known to possess gross amounts of residual cortical tissue, eight survived indefinitely and the remaining animal died within 24 hours of the operation. This death was attributed to the effects of post-operative shock. Of the seventy-nine mice known not to possess gross amounts of adrenal tissue seventy-one died and in fifty-eight of these the cause of death was assumed to be the development of adrenal insufficiency. Indefinite survival in eight mice was attributed to escape of microscopic amounts of cortical

tissue after puncture of the adrenal capsule. Macroscopic examination of the operative site did not, however, reveal the presence of cortical tissue in these mice.

Neither sex, nor the addition of 1 per cent of sodium chloride to the drinking water was shown to affect the ability of adrenalectomised mice to survive. The survival period of female mice was slightly, but not significantly, longer than that of male mice.

B. THE EFFECT OF AGE, SEX, AND SALINE ADMINISTRATION UPON THE REACTION OF ALBINO MICE TO ADRENALECTOMY

In view of the partly unexpected results obtained in the preliminary experiment, it was decided to re-investigate the effects of sex and of sodium chloride administration in immature mice. It was further decided to examine the effect of these factors on the survival of mature mice adrenalectomised at six or twelve weeks of age. The inclusion of mice at three ages enabled the consideration of the effect of age on the response of mice to adrenalectomy. The effect of age had not previously been investigated in mice.

Materials and Methods

Animals

Male and female mice of the albino strain described in the previous section were used. Operations were performed within three days of weaning (21 days from birth), at 40 to 45 days of age, or at 85 to 90 days.

Diet

The diet, fed ad libitum, was as previously described in the first section of this chapter.

Operative Techniques

Adrenalectomy was carried out in the manner described in the first section of this chapter. In the older animals a considerable bulk of adipose tissue was removed together with the adrenal. Gonadectomy was carried out at the same time as adrenalectomy.

In the female the ovaries were exteriorised through the body wall incisions made in the course of adrenalectomy. The ovary, fallopian tube

and a portion of the uterine horn were removed by cauterisation. The combined operation--adrenalectomy plus ovariectomy--took very little more time to perform than simple adrenalectomy.

Castration in the male involved entering the body cavity from the ventral surface. The skin and body wall incisions were made slightly anterior to the genital papilla; the testes exteriorised through the incision and removed by cauterisation. In the immature male castration was not difficult, but the extra time during which the animal was under anaesthesia appeared to be a cause of a greater degree of post-operative shock in the doubly operated male. In the adult male the operation was hindered by a considerable amount of adipose tissue, which surrounded the testes, to an extent that the total operative time for the removal of both adrenals and testes was prohibitive. Castration was not persisted with in the mature males.

It was found that the mature mice were more sensitive to the effects of prolonged anaesthesia than the immature mice. In particular, the animals operated at 12 weeks of age were prone to die in the course of the operation, where this was prolonged beyond ten minutes, or where the depth of anaesthesia was other than shallow.

Application of Treatments

Within each age group animals of each sex were allocated at random to treatments arranged according to a 2 x 2 factorial design. Level of gonad activity--presence or absence of gonads--and level of added salt--presence or absence of 1 per cent of added sodium chloride in the drinking fluid--formed the treatments. In the two mature age groups, castrate male mice were not included.

In the three and six weeks of age series, ten animals of each sex were included in each individual treatment while in the twelve weeks of age series each treatment group contained seven animals of each sex. The data presented in this section was thus derived from 80 mice adrenalectomised at three weeks of age, 60 mice operated at six weeks, and 42 mice operated at twelve weeks.

Exclusion of Data

Animals in which completeness of adrenalectomy was suspect were noted and the data regarding any of these that survived indefinitely were excluded. Similarly data pertaining to mice which died within 36 hours of the operation were omitted. The mice thus excluded from consideration were replaced by animals selected at random. This procedure disturbed to some extent the relation of replications to "time changes". It was considered, nevertheless, that the analysis of variance technique would still allow the segregation, as the variance due to differences between the block means, of most of the effect of changes during the course of the experiment.

Post-Operative Care; Recording

The post-operative care was as previously described. The records kept and the manner of recording were the same as in the preliminary investigation.

TABLES V, VI, VII.

TABLE V

THE EFFECT OF AGE, SEX, GONADECTOMY, AND SODIUM CHLORIDE ADMINISTRATION UPON THE MEAN SURVIVAL PERIOD OF ADRENALECTOMISED ALBINO MICE

		AGE AT OPERATION 21-25 DAYS			AGE AT OPERATION 40-45 DAYS			AGE AT OPERATION 84-90 DAYS		
		No.	Mean Days	Mean Log. Days	No.	Mean Days	Mean Log. Days	No.	Mean Days	Mean Log. Days
A. INDIVIDUAL TREATMENT MEANS										
WATER	FEMALE	10	4.8	0.648	10	4.6	0.643	7	6.0	0.691
	SPAYED FEMALE	10	2.9	0.449	10	5.1	0.654	7	6.4	0.722
	MALE	10	3.0	0.464	10	4.5	0.645	7	6.9	0.812
	CASTRATE MALE	10	3.7	0.534						
SALINE	FEMALE	10	4.5	0.633	10	6.3	0.771	7	8.0	0.873
	SPAYED FEMALE	10	3.1	0.443	10	4.8	0.662	7	6.7	0.772
	MALE	10	3.0	0.454	10	5.2	0.691	7	7.1	0.780
	CASTRATE MALE	10	3.1	0.469						
B. POOLED TREATMENT MEANS										
WATER		40	3.60	0.524	30	4.73	0.647	21	6.43	0.742
SALINE		40	3.43	0.500	30	5.43	0.708	21	7.29	0.809
	FEMALE	20	4.65	0.640	20	5.45	0.706	14	7.00	0.782
	SPAYED FEMALE	20	3.00	0.446	20	4.95	0.658	14	6.57	0.747
	MALE	20	3.00	0.459	20	4.85	0.668	14	7.00	0.796
	CASTRATE MALE	20	3.40	0.501						
SIGNIFICANCE OF DIFFERENCES[1.]										
SALINE v. WATER				N.S.			N.S.			N.S.
BETWEEN SEX GROUPS				**			N.S.			N.S.
MALE v. FEMALE				*			--			--
BETWEEN MALE GROUPS				N.S.			--			--
BETWEEN FEMALE GROUPS				**			--			--
C. POOLED AGE MEANS		80	3.51	0.512	60	5.08	0.677	42	6.86	0.775
SIGNIFICANCE OF DIFFERENCES[2.]										
ORIGINAL DATA		Highly significant** difference between 21-25 and 40-45 days age groups; significant* difference between 40-45 and 84-90 age groups.								
TRANSFORMED DATA (Log.)		Highly significant** differences between 21-25 and 40-45 age groups, and between 40-45 and 84-90 age groups.								

N.S.: Non-significant difference ($p > 0.05$); -- : Not tested; $*p < 0.05$; $**p < 0.01$

1. Differences between the means examined by "F" test (see Table XV) on the transformed data;

2. Differences between the means examined by approximate "t" test (see text) employing the residual variance (see Table XV) in the case of the transformed data and the total variance in the case of the original data.

TABLE VI

TIME CHANGES IN THE MEAN SURVIVAL PERIOD OF ADRENALECTOMISED ALBINO MICE

	AGE AT TIME OF OPERATION							
	21-25 DAYS Saline		21-25 DAYS Water		40-45 DAYS		84-90 DAYS	
	No.[3.]	Mean[1.]	No.[3.]	Mean[1.]	No.[3.]	Mean[2.]	No.[3.]	Mean[2.]
B	5	0.345	2	0.389	4	0.506	4	0.593
L	6	0.389	8	0.433	1	0.583	2	0.622
O	10	0.400	3		3	0.630	1	0.648
C	3	0.420	4	0.464	5	0.659	3	0.683
K	9	0.464	6		9	0.672	5	0.823
A	7	0.527	7		2	0.692	7	1.001
R	2	0.551	5	0.584	8	0.714	6	1.056
R	4	0.564	10		7	0.750		
A	8	0.588	9	0.644	6	0.757		
Y	1	0.748	1	0.748	10	0.813		

1. Four mice in each mean; 2. Six mice in each mean; 3. Blocks numbered in time sequence.

Note: Any two means not joined by the same line are significantly different. Any two means joined by the same line are not significantly different ($p > 0.05$).

TABLE VII

THE FREQUENCY DISTRIBUTION OF DEATHS OF ADRENALECTOMISED ALBINO MICE: ESTIMATES OF INDICES OF SKEWNESS AND KURTOSIS

	IMMATURE MICE[1.] (n = 138)		MATURE MICE[2.] (n = 102)	
	g_1	g_2	g_1	g_2
ORIGINAL DATA	0.936***	0.118[=]	1.448***	2.178***
TRANSFORMED DATA	0.206[-]	-0.410[+]	0.217[-]	0.510[-]

1. Includes mice from the preliminary investigation together with those operated at 21-25 days in the present investigation

2. Includes mice operated at six and twelve weeks of age.

***$p < 0.001$; [+] $0.10 > p > 0.05$; [-] $0.50 > p > 0.10$; [=] $p > 0.50$

The values of p shown represent the probabilities of the observed deviations of g_1 and g_2 from the expected values for a normal distribution (zero in each case), being due to chance.

Results

Mortality Rate

All mice in which the capsule of the removed adrenals appeared to be intact died. Of 18 animals in which the integrity of the capsule of one of the glands was suspect, nine survived indefinitely. The distribution of these indefinite survivors between the various treatment groups and ages appeared to be at random.

Deaths due to Post-Operative Shock

On the basis of the criteria suggested by the results of the preliminary investigation a total of 14 mice were considered to have succumbed to the effects of shock. The longest survival of any mouse included in this group was between 18 and 24 hours. In the two mature age series the distribution of deaths caused by post-operative shock showed no relation to the treatments. In the immature age group, however, five of a total of seven animals dying within 36 hours of the operation were castrate-adrenalectomised males. This was considered an effect of the more extended period of anaesthesia. If the group of castrate males was ignored (this group was not repeated in the mature age series) there was no evidence that the proportion of animals dying within 36 hours in the immature age group (2) was greater than in the 42 day-old (3) or 84 day-old (4) age groups.

Factors Affecting the Survival Period

The mean survival times, and tests of significance of the differences between these means, for the various age and treatment groups are shown in Table V. The analyses of variance (Snedecor, 1946, pp 304 et sqq.) are shown in Table XV.

It was necessary, for the valid application of the analysis of variance,

to transform the data for each age group to a scale on which heterogeneity among sub-class variances was minimised. The use of a logarithmic metameter was suggested by an apparent relation between the sub-class means and variances.

In a later section of these results the frequency distribution for each age group has been examined, and it has been suggested that the population of survival periods for each age group was more satisfactorily described by a "log-normal" distribution than by a normal distribution. The success of the logarithmic transformation in stablising the sub-class variances in part reflected the nature of the distribution describing the population for each age group.

The Effect of Age on the Survival Period

The means for each age series are shown in Table V together with the significance of the differences between these means. The procedure used to examine these differences was an approximate "t" test appropriate in the situation where the variances and numbers of observations differ between the "treatments" (Cochran and Cox, 1950, pp 91 et seq.). This examination was carried out on both the original and the transformed scale. In each case the conclusion drawn was that the mean for the six weeks of age group differed from the means for the other two age groups. The animals operated at three weeks of age survived adrenalectomy on average for a shorter period than those operated at six weeks, and these in turn survived for a shorter time than mice operated at twelve weeks of age.

The Effect of Sex and Gonadectomy

In the animals operated within a few days of weaning a statistically significant effect of the ovaries could be demonstrated. This was reflected

in the overall sex difference and in the greater survival period of the adrenalectomised female over that of the adrenalectomised-ovariectomised female (see Table V). This latter difference contrasted with the lack of significant effect of castration in the male.

In both older age groups there were no significant differences between the sexes, or between ovariectomised females, and females possessing ovaries. In the six-weeks-old group, however, the females survived on average slightly longer than males or ovariectomised females.

The Effect of Substitution of Saline for Water

No significant differences could be demonstrated between the mean survival periods of mice receiving the two drinking fluids--tap water or tap water plus 1 per cent of sodium chloride--in any of the three age series. In both mature age groups, the animals given saline had a slightly greater mean survival period than those given water. In the case of the mice operated at 42 days of age this difference approached significance ($p < 0.10$).

Variation of Survival Periods with Time

In all age groups there was evidence of changes in the survival time over the period covered by the experiment. Such changes were reflected in the statistically significant interaction between blocks and drinking solutions found in the immature mice and in significant differences between the block means within the mature age series.

The appropriate means (i.e. block means for each drinking solution, or overall block means) are shown, together with the results of statistical examination, in Table VI. The procedure employed to detect the whereabouts of significant differences in the array of means was the multiple range test described by Duncan (1955). The examination was conducted on means computed

on the logarithmic scale.

In the three weeks of age series, examination of the means for the four mice in each block receiving each of the two drinking solutions did not reveal any systematic change in survival period with time. Similar examination of the means for the six animals in each of the replications within the six weeks of age series also failed to reveal any systematic changes. In the twelve weeks of age group, the first four block means differed significantly from the last two block means, but this could not be considered evidence of a regular time-change in survival period.

The absence of a systematic increase in survival period with time, in any age group, was taken to indicate that the significant differences between blocks did not reflect a gradual improvement in operative technique. It did not necessarily follow that such improvement had not occurred.

The Nature of the Frequency Distribution of Survival Periods

The frequency distributions of the time of death after adrenalectomy, for the three age groups, are shown in Fig. 2.

The nature of the distribution of survival periods for mature and immature mice has been examined statistically, on both the original and transformed scales, by computing the indices of skewness (g_1) and kurtosis (g_2) (Snedecor, 1946, pp 176 et seq.). In the case of mice adrenalectomised within a few days of weaning, the results of the preliminary investigation have been combined with those reported in this section to give a total sample size of 138 observations. The numbers in each of the two mature age series were too small for dependable estimation of g_1 and g_2, and estimates of these paramaters were computed from a sample of 102 observations, obtained by combining the results for the six and twelve weeks of age series. The estimates of g_1 and g_2, together with the results of tests of significance (employing the "t" test) of the

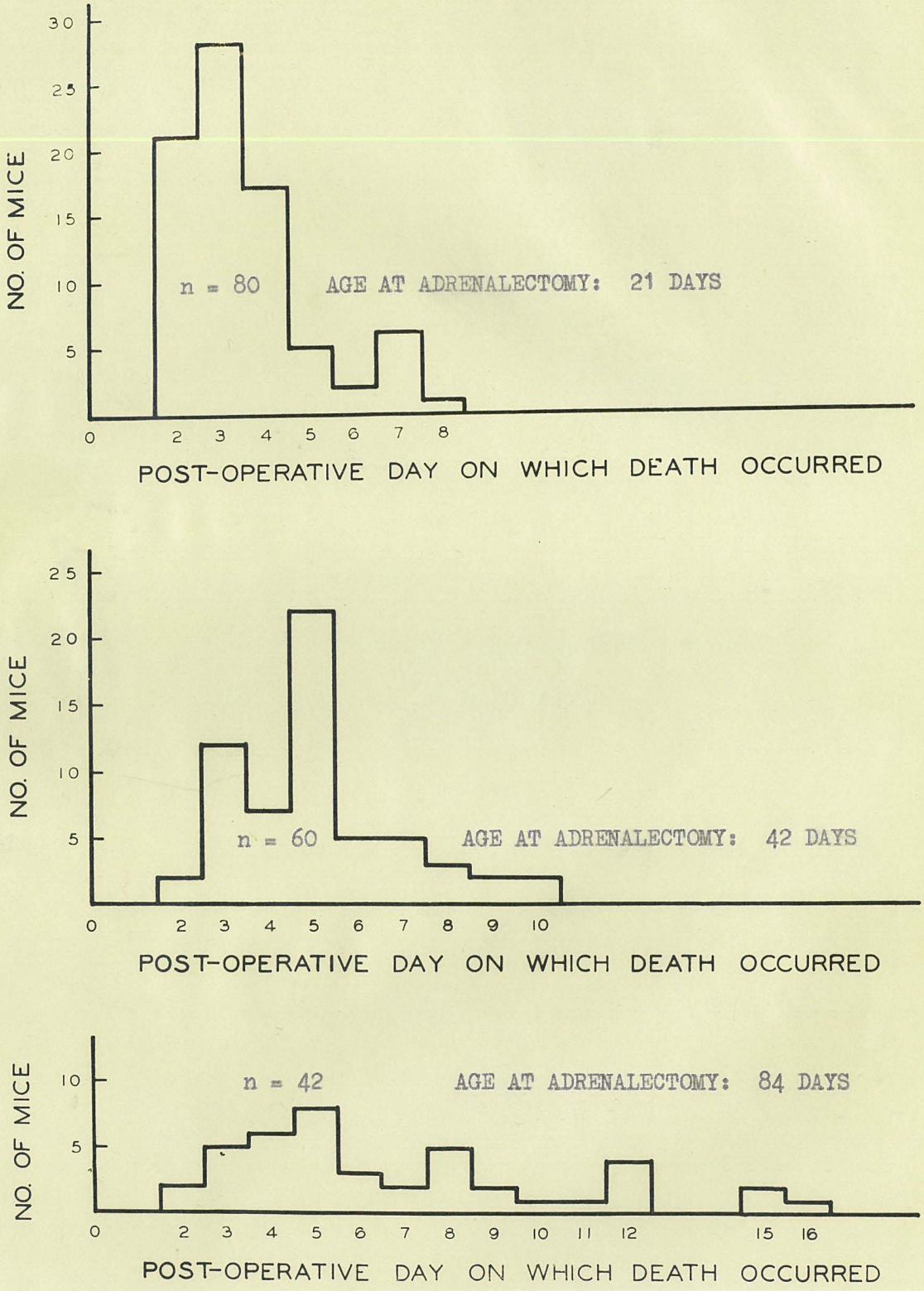

FIGURE 2. FREQUENCY DISTRIBUTION OF THE LIFESPAN OF ADRENALECTOMISED ALBINO MICE.

differences between these estimates and the values characteristic of the normal distribution, are presented in Table VII.

On the original scale, the distributions for both mature and immature mice were highly significantly different from the normal distribution as regards skewness. When transformed to a logarithmic scale, neither distribution differed significantly from a normal distribution in this regard. The distribution for immature mice was not significantly peaked on the original scale, whereas that for mature mice showed a highly significant degree of kurtosis. On a logarithmic scale the distribution for immature mice had a negative degree of kurtosis approaching significance while that of the distribution for mature mice did not differ significantly from zero. It was concluded that, although the distribution in the case of immature mice was more nearly normal on the logarithmic scale than on the original scale, it was not sufficiently peaked on the latter scale for the population of survival periods to be adequately described by a "log-normal" (Bliss and Calhoun, 1954) distribution. On the other hand, the estimates of the population parameters obtained, on the two scales, from the sample distribution for mature mice indicated that the population could adequately be described by a "log-normal" distribution.

Because of the composite nature of the sample on which it was based, the conclusion regarding the nature of the distribution of survival periods for mature mice had to be qualified. The means and variances of the separate distributions for the two mature age groups differed on both scales. The conclusion regarding the population of survival periods for mature mice (of the given strain) could be sustained with reference to a population of these two ages, but not with reference to a sub-population for mature mice of either particular age. Further, this conclusion could be sustained for mature mice generally, only if the samples from the two sub-populations could be regarded collectively as a random sample from the population of survival periods for

mice of all mature ages.

The validity of the assumptions, necessary if the conclusion regarding the distribution of the survival period of mature adrenalectomised albino mice was to be sustained, could be doubted. Accordingly the nature of the distribution for each mature age group was examined separately. The technique employed has been described by Bliss and Calhoun, (1954) (pp. 48 et seq.) for use with samples of 30 or more observations. The results of the application of this technique are shown graphically in Fig. 3.

The probits of the cumulative distribution for each age group, on each of the original and transformed scales, were compared graphically with the corresponding theoretical normal distribution described by the sample mean and variance. Inspection of the resulting graphs indicated that, in both age groups, the observed sample distribution was more closely approximated on the logarithmic scale by the theoretical linear form of the normal distribution. It was concluded that the two age populations of survival periods, for mature mice of the given strain, were adequately described by log-normal distributions.

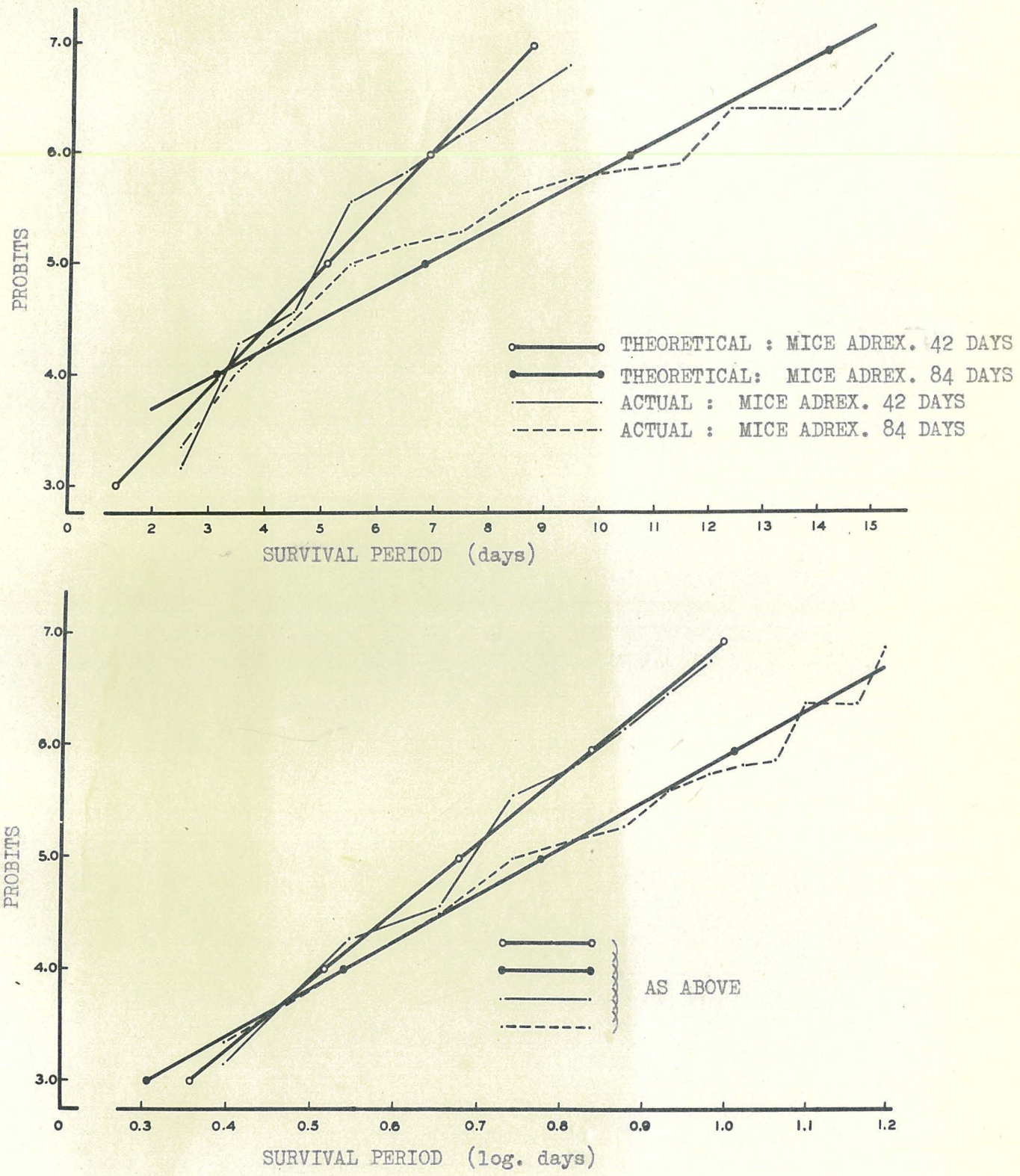

FIGURE 3. THE PROBIT FORM OF THE CUMULATIVE DISTRIBUTION OF THE LIFESPAN OF MICE ADRENALECTOMISED AT 42 OR 84 DAYS OF AGE: DEVIATION FROM NORMALITY ON THE ORIGINAL AND TRANSFORMED SCALES.

Discussion

Indefinite Survivals

The absence of indefinite survival in those mice in which the adrenals were removed without damage to the capsule confirmed and extended the earlier conclusion that accessory cortices were not functionally important in immature mice of the strain investigated. A similar absence of functional accessory cortical tissue was reported by Howard (1946), with immature mice of the H strain, and by Hill (1948), with mature mice of the CHI and Swiss albino strains. Other workers have reported the apparent presence of functional adrenal rests in a proportion of mature (Dorfman, et al., 1946; Masui, 1928) and immature (Masui, 1928) adrenalectomised mice.

The Influence of Post-Operative Shock

The distribution of deaths occurring within 36 hours of adrenalectomy at each of the three ages was such as to confirm the hypothesis, advanced in a previous section of this chapter (p44), that exclusion of data from adrenalectomised mice with lifespans of less than 36 hours minimised the effect of post-operative shock. The longest survival in this group of mice fell short of the dividing line--between death due to shock and death as a result of adrenal insufficiency--by more than 12 hours.

The proportion of short survivals in the present experiment (7.1 per cent) was less than half that obtained in the preliminary investigation. Pfeiffer and Hooker (1940), who indicated that post-operative shock was of importance in mature mice of the A strain, reported a similar decrease in the proportion of short survivals as the operator became more practiced in the technique of adrenalectomy. The decreased proportion of deaths due to operative shock in the present investigation was obtained in spite of the inclusion of mature mice adrenalectomised at 12 weeks of age which were deleteriously affected by more than a very

short period of ether anaesthesia.

The most important component cause of post-operative shock in the strain of mice used in this investigation appeared to be the degree and duration of anaesthesia. Firor and Grollman (1933) were likewise of the opinion that the deleterious effects of anaesthesia were important in mice; these were apparently of considerable importance since approximately half of the mature mice adrenalectomised by these workers died within 24 hours of operation. Nissim (1953) considered that xylocaine was preferable to ether as an anaesthetic for mice, however, Hill (1948) obtained satisfactory results with ether in adult CHI mice provided the period of anaesthesia was not unduly long.

Factors Affecting the Survival Period

Addition of Sodium Chloride to the Drinking Water. The addition of 1 per cent sodium chloride to the drinking water was without significant effect on the survival period of mice adrenalectomised at all three ages. Those mice adrenalectomised at 42 days from birth that were provided with saline drinking fluid lived on average almost a day longer than similar mice given tap water to drink, this small difference approached statistical significance ($p<0.1$), at the 5 per cent level.

A similar absence of any notable effect of the addition of sodium chloride to the drinking solution was observed in mature adrenalectomised mice of the A strain (Pfeiffer and Hooker, 1940), the Swiss albino strain and CHI strain (Hill, 1948). Howard (1946) was able to maintain about a third of immature adrenalectomised mice of the H strain by a similar procedure; more uniform results were obtained by feeding a high-sodium, low-potassium diet. Marder (1950) claimed almost total protection of mature adrenalectomised mice of the CBA strain fed a diet of bread and milk and provided with a 1 per cent sodium chloride drinking solution, but a contributory effect of accessory cortical tissue

could not be excluded in these mice. Mice of the A and C57 (black) strains did not respond to this regimen (Marder, 1950).

A considerable degree of variation--both between strains and between individual animals from the same colony--in the effectiveness of added sodium chloride has been reported with the rat (Cowie, 1949b; Gaunt, et al., 1935; Grollman, 1941; Ingle, et al., 1943; Kutz, et al., 1934). In general, however, the simple expedient of the addition of sodium chloride to the drinking water seemed more effective in rats than in mice where notable results were obtained (Howard, 1946; Marder, 1950) only with regimen in which the level of potassium in the diet was restricted. The potassium content of the diet used in the present investigations was not determined, but was unlikely that this--on the basis of the mineral content of materials similar to the components (Morrison, 1949)--was greater than 1 per cent (K_2O) by weight on a moisture free basis. In any event, since the control of dietary levels of sodium and potassium did not suffice to alleviate adrenal insufficiency in mice of the A and C57 (black) strains (Marder, 1950), it did not necessarily follow that a diet lower in potassium would have aided survival in adrenalectomised albino mice.

Age at Adrenalectomy.

The mean survival period of albino mice increased with age at adrenalectomy (Table V). A similar stepwise age-increase in survival time was reported with hooded Norway rats by Cowie (1949a) (Fig. 4); at corresponding ages the survival periods of rats were approximately twice as long as those of mice. Other workers have indicated that rats adrenalectomised prior to puberty lived for shorter periods than sexually mature rats (Firor and Grollman, 1933; Sisson and March, 1935).

There did not appear to be any previously reported comparison of the life-

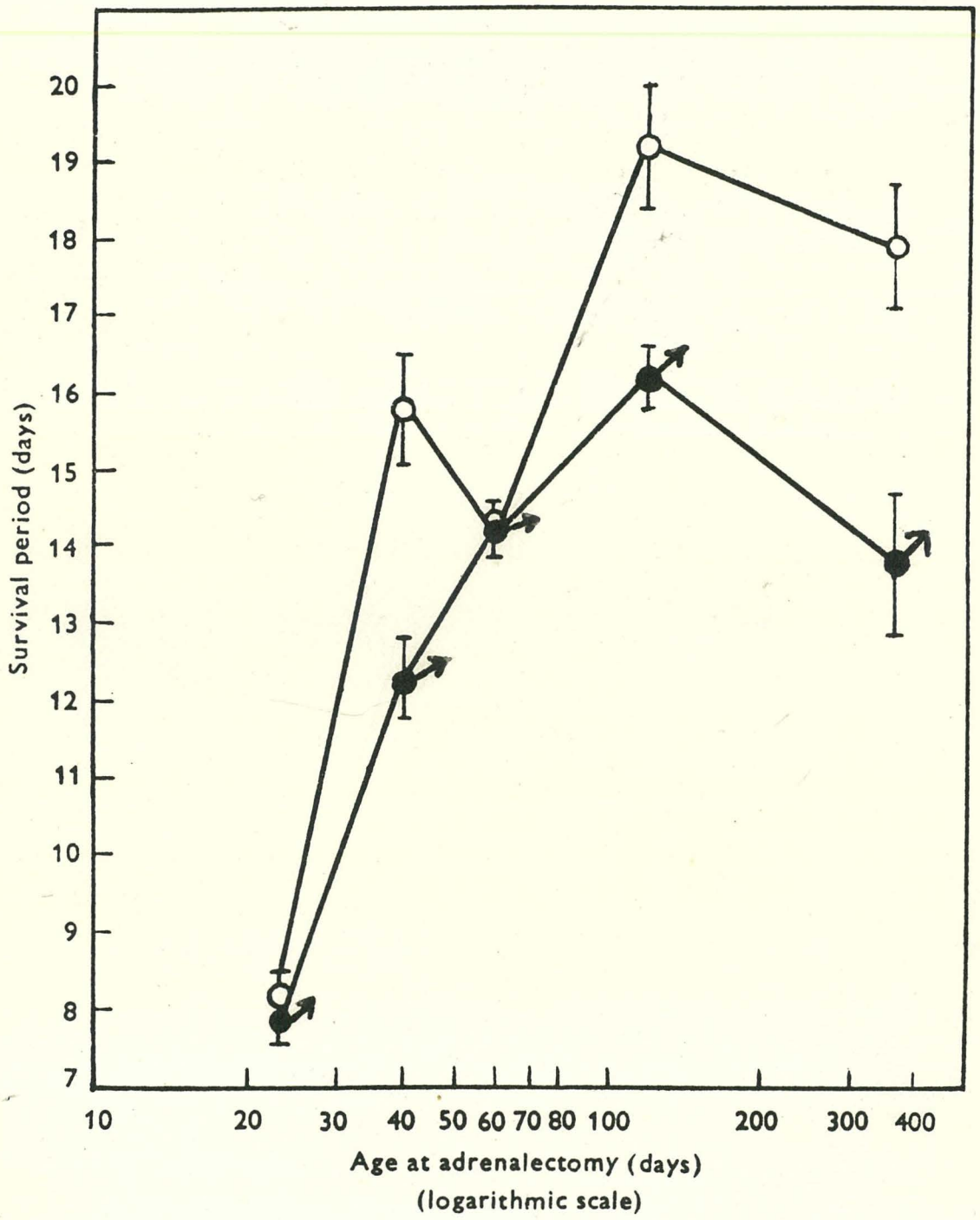

FIGURE 4. THE RELATION BETWEEN MEAN LIFESPAN AND AGE AT THE TIME OF ADRENALECTOMY IN THE HOODED NORWAY RAT (From Cowie, 1949a).

Male (♂) and female rats are shown separately and the standard errors of the means are indicated by the vertical lines passing through each point.

span of mice adrenalectomised at different ages. The survival periods of mature mice adrenalectomised in the present study were very similar to those reported previously for mice of similar ages (Firor and Grollman, 1933; Masui, 1928; Pfeiffer and Hooker, 1940), but were noticeably less than those obtained by Hill (1948) with intact CHI and Swiss albino mice adrenalectomised at ages between 100 and 200 days. Howard (1946) reported a peak period of mortality at about the fourth day after adrenalectomy in a small number of immature mice of the H strain. This seemed to indicate a rather similar mean survival period to that obtained with albino mice adrenalectomised at a similar age in the present and preliminary investigations.

Sex and Gonadectomy.

In the present experiment the ovaries of immature female mice appeared to have a small but significant survival value. This finding agreed with the small non-significant difference between the sexes obtained in the preliminary study. A similar effect of the ovary could not, however, be demonstrated in mice adrenalectomised at sexually mature ages.

An effect of the immature ovary upon the lifespan of adrenalectomised females had not previously been reported. Hill (1948) observed, but dismissed as undependable, a reduced survival time in ovariectomised mature mice of the Swiss albino strain. Kroc (1938) detected no difference in the period of survival after adrenalectomy between ovariectomised and non-ovariectomised rats of the Sprague-Dawley strain, whereas Snyder and Wyman (1951) found that the ovary of mature hamsters had a marked survival value.

Comparisons of the survival periods of mature male and female animals have been made by a number of workers. The sex of the adrenalectomised animal was without effect on the lifespan in CHI mice (Hill, 1948) and in rats of Wistar origin (Cleghorn, et al., 1936; Gaunt, 1933; Schultzer, 1935). The female

was reported to outlive the male after adrenalectomy in the hooded Norway rat (Cowie, 1949a) and hamster (Snyder and Wyman, 1951); the converse appeared to be the case with an albino strain of rats (McKeown and Spurrel, 1940).

It was suggested (Cf. ante, pp. 18 et sqq.) that conflicting reports of the effect of ovariectomy, and of the effect of the sex of the adrenalectomised animal might be reconciled in terms of the reaction of the ovary to adrenal insufficiency. In view of the established corticomimetic activity of progesterone (Bourne, 1939; Emery and Greco, 1940; Gaunt and Hays, 1938; Gaunt, et al., 1938; Greene, et al., 1939; Schwabe and Emery, 1939; Snyder and Wyman, 1951; Wells and Greene, 1939); the effect of the luteinised ovary (D'Amour and D'Amour, 1939; Emery and Greco, 1940; Pfeiffer and Hooker, 1940), and the association of extended survival with marked lengthening of the dioestrous stage of the oestrous cycle (Snyder and Wyman, 1951; Wyman, 1928) it was supposed that, when the ovarian reaction involved the secretion of effective levels of progesterone in the majority of adrenalectomised females, a favourable effect of the ovary on survival might be obtained. When an effective level of oestrogen--sufficient to exert a deleterious effect (Gaunt and Hays, 1938; Gaunt, et al., 1938; Pfeiffer and Hooker, 1940) upon the adrenalectomised animal-- was secreted by the ovary of the adrenalectomised female, the mean survival period of the male would be expected to exceed that of the female. Where neither ovarian reaction was dominant no net survival value of the ovary would be apparent.

The greater survival of the ovary-intact albino mice adrenalectomised just prior to puberty, in the present experiment, contrasted with the absence of any effect of sex upon the lifespan of hooded Norway rats adrenalectomised at a similar age (Cowie, 1949a). On the other hand, female hooded Norway rats adrenalectomised at mature ages survived longer than male rats (Cowie, 1949a)

while in the present investigation sex and gonadectomy had no significant effect upon the lifespan of mature adrenalectomised mice.

The increased lifespan of the ovary-intact immature mice could have been due to the production of an ephemeral but effective level of progesterone by the ovary. This assumed secretory ability of the immediate pre-pubertal mouse ovary was supported by the results of gonadotrophic stimulation in 21 day old mice of the A strain (Greene, 1955). A single subcutaneous injection of pregnant mare's serum (PMS) caused the secretion of progesterone in demonstrable amounts within 12 hours; detectable effects of oestrogen secretion were not apparent till about 24 hours after PMS treatment.

The findings of Clayton and Prunty (1951) (Cf. ante. p.20) indicated that the agent stimulating the postulated release of progesterone from the ovary in adrenalectomised animals might have been ACTH. Administration of ACTH to intact mature CHI mice was found to cause ovarian and vaginal changes suggestive of progesterone secretion (Flux and Munford, unpublished). Clayton and Prunty (1951) found that there was a seasonal variation in the responsiveness of the ovary to ACTH stimulation which could be related to the functional state of the ovary. A difference in the sensitivities of mature and immature ovaries to endogenous ACTH might have accounted for the postulated failure of the former to produce effective levels of progesterone. On the other hand the level of endogenous ACTH produced as a result of adrenalectomy might have been lower in mature animals.

The postulated ability of the immature and mature ovaries to secrete effective levels of progesterone were not necessarily diametrically different in hooded Norway rats and albino mice. The immature rats adrenalectomised by Cowie (1949a) were further removed from puberty than the immature mice operated in the present experiment. Immature hooded Norway rats adrenalectomised immed-

iately prior to puberty may have shown a sex difference in post-operative lifespan.

The Frequency Distribution of Survival Period

The lifespans of albino mice adrenalectomised at six or twelve weeks of age appeared to be distributed log-normally; the survival periods of mice adrenalectomis at three weeks of age were more adequately described by a log-normal than by a normal distribution. Hemmingson (1938) likewise concluded that the logarithms of the survival periods of rats, cats and Addisonian patients were normally distributed. This worker used a graphical technique to examine data obtained with rats in his own laboratory, and reported by other workers with rats (Gaunt, 1933), cats (Marine and Baumann, 1927) and Addisonian patients (Guttman, 1930). Cowie (1949a) computed the indices of skewness and kurtosis from the sample distributions of the lifespan of rats of each sex adrenalectomised at a number of ages. The sample sizes were too small for precise estimation of these indices, but each mature age one or both of the male and female distributions departed significantly from normality. The nature of these deviations from the normal distribution suggested that the lifespan of mature adrenalectomised rats was described by a log-normal distribution. In the case of immature rats, however, neither index differed significantly from the normal value in either sex. This may have in part reflected the small sample sizes and consequent low precision of the estimates, but was consistent with the conclusion, that in immature mice the distribution of lifespan was less markedly non-normal than in mature mice, reached in the present investigation.

Time Changes in Survival Period

The lifespans of mice adrenalectomised at each of the three ages appeared to vary quite markedly with time in the present investigation. These changes could not be related to any known changes either in the post-operative conditions, operative technique or in the breeding colony. Similar inexplicable changes in a number of characteristics have been often reported with colonies of small animals. In particular quite marked variation in the survival periods of rats from the same colony adrenalectomised at different times have been reported by Cowie (1949a), Gaunt, et al. (1935) and Cleghorn, et al. (1936).

Summary

Total adrenal ablation was followed by death in all albino mice operated at each of three, six or twelve weeks of age.

The mean survival period of adrenalectomised mice increased with age at operation.

In female mice adrenalectomised prior to puberty the ovaries appeared to promote a slight but significantly increase in lifespan. No effect of sex or of the removal of the ovaries could be demonstrated in mice adrenalectomised at either six or twelve weeks of age. Castration in mature and immature male mice did not appear to affect the time for which the animals survived adrenalectomy.

The addition of 1 per cent sodium chloride to the normal drinking fluid (tapwater) was without significant effect upon the lifespan of mice adrenalectomised at any of the three ages.

There was evidence of changes in the response of mice to adrenalectomy with time. These changes, reflected in significant differences between the block means for survival time, did not appear to be related to any known changes in the colony, in post-operative treatment or in the operative technique.

The nature of the frequency distribution of the lifespan of mice adrenalectomised at each age was examined statistically. It was concluded that whereas the survival periods of each of the two mature age groups were adequately described by a log-normal distribution, the distribution for the immature mice though non-normal, was not necessarily log-normal.

C. A PRELIMINARY STUDY OF THE RESPONSE OF MICE OF THE AW, CBA, AND NZC STRAINS TO BILATERAL ADRENALECTOMY

The results obtained with albino mice indicated that these animals could not be protected against the fatal effect of adrenalectomy by the simple expedient of adding 1 per cent of sodium chloride to the drinking water. Mice of this albino colony were not suitable, therefore, for an investigation of the effect of total adrenal insufficiency upon the mammary gland. Mice of three other strains were available in small numbers and these were utilised in preliminary studies of the effect of increased sodium chloride intake upon lifespan after adrenalectomy.

Materials and Methods

Animals

Twenty-six male mice of the NZC strain were adrenalectomised at ages between 21 and 35 days. These mice were obtained from an inbred colony maintained by Dr. D.S. Flux at Massey Agricultural College. This colony was established from nine breeding mice provided in November, 1954 by Mr. W.H. Hall, Director of the Animal Department, Faculty of Medicine, University of Otago. The NZC was one of a number of inbred strains bred from a stock of piebald mice by Dr. Marianne Bielschowski, Department of Cancer Research, Faculty of Medicine, University of Otago. The NZC mice used in this study were the offspring of animals which had been inbred (brother-sister mating) for 27 or 28 generations.

Eighteen male and sixteen female mice of the AW strain, and ten male and nine female mice of the CBA strain were adrenalectomised at ages between 40 and 48 days. These mice were obtained from colonies maintained by Mr. F.R. Cockrem at Massey Agricultural College. The foundation stock for these colonies were supplied in 1955 by Dr. A.S. Fraser of the Animal Genetics Section, C.S.I.R.O., University of Sydney. Information regarding the genetic origin of the AW strain was not

TABLES, VIII, IX, X.

TABLE VIII

OCCURRENCE OF DEATH OR INDEFINITE SURVIVAL IN ADRENALECTOMISED MICE OF THE CBA, AW, AND NZC STRAINS

A. DISTRIBUTION OF MICE AMONG THE SEVERAL SURVIVAL CATEGORIES

	C B A				A W				N Z C	
	WATER		SALINE		WATER		SALINE		WATER	SALINE
	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	MALE	MALE
DYING WITHIN 36 HRS	-	-	-	-	-	2	-	1	-	2
BEFORE 21 DAYS*	5	5	3	4	8	4	6	2	15	9
AFTER 21 DAYS**	-	-	1	1	1	3	1	3	-	-
NOT DYING	-	-	-	-	1	3	1	3	-	-

B. EXAMINATION OF DIFFERENCES**

Differences in the Occurrence of Indefinite Survival

AW Mice	Female	Male	Total	Saline	Water	Total
Dying After 36 hrs	14	9	23	11	12	23
Not Dying	2	6	8	4	4	8
Total	16	15	31	15	16	31
	p = 0.078					

Differences in the Occurrence of Prolonged Survival

CBA Mice	Female	Male	Total	Saline	Water	Total
Dying Before 21 days*	8	9	17	7	10	17
Dying After 21 days	1	1	2	2	0	2
Total	9	10	19	9	10	19
				p = 0.206		

AW Mice	Female Saline	Male Saline	Total Saline	Saline Male	Water Male	Total Male
Dying Before 21 days*	6	2	8	2	4	6
Dying After 21 days	0	3	3	3	0	3
Total	6	5	11	5	4	9
	p = 0.119			p = 0.093		

*Exclusive of mice dying before thirty-six hours.
**Includes those mice which survived during the period when sodium chloride was added to the drinking water, but died after this was discontinued.
**Probabilities that the discrepancies in the 2x2 contingency were due to chance were computed by Fisher's exact method (Goulden, 1952, pp. 372 et sqq.).

TABLE IX

MEAN SURVIVAL PERIODS* OF CBA, AW, AND NZC MICE DYING WITHIN TWENTY-ONE DAYS OF BILATERAL ADRENALECTOMY
(Mice dying within the first thirty-six hours are excluded.)

	C B A				A W				N Z C	
	WATER		SALINE		WATER		SALINE		WATER	SALINE
	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	MALE	MALE
SEX within DRINKING SLN	7.4 ±0.4**	10.2 ±1.9	12.0 ±2.1	12.2 ±0.8	11.6 ±1.4	5.2 ±1.0	5.7 ±1.3	9.5 ±4.5	3.9 ±0.4	4.9 ±0.9
DRINKING SLN w. STRAIN	8.8 ± 1.0		12.1 ± 0.9		9.5 ± 1.4		6.6 ± 1.1		3.9 ±0.4	4.9 ±0.9
STRAIN	10.2 ± 0.8				8.4 ± 1.0				4.2 ± 0.4	

* in days **Standard error of the mean.

TABLE X

MEAN LOSS IN BODY WEIGHT* OF CBA AND AW MICE DYING WITHIN TWENTY-ONE DAYS OF BILATERAL ADRENALECTOMY
(Mice dying within the first thirty-six hours are excluded.)

	C B A				A W			
	WATER		SALINE		WATER		SALINE	
	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE
SEX within DRINKING SOLN	2.80 ±0.21**	3.78 ±0.60	2.27 ±0.58	4.60 ±0.39	3.59 ±0.30	3.25 ±0.23	3.48 ±0.28	3.25 ±0.85
DRINKING SOLN w. STRAIN	3.29 ± 0.53		3.60 ± 0.56		3.47 ± 0.21		3.42 ± 0.26	
STRAIN	3.42 ± 0.30				3.45 ± 0.08			

* in gms **Standard error of the mean.

available to the present investigator at the time of writing. The origin of the CBA strain has been described by Strong (1942).

Experimental Procedure

As mice became available they were adrenalectomised and allotted at random to one of two treatment groups. Mice in one group were given tap water to which 1 per cent of sodium chloride had been added as a drinking solution; mice in the other group were given tap water to drink. Mice given the saline drinking fluid that did not die prior to the twenty-first post-operative day had tap water substituted for the saline solution on this day.

The diet, environmental temperature, and general post-operative conditions were similar to those obtained in earlier experiments with albino mice. The effect of adrenalectomy upon body weight and upon changes in the vaginal epithelium were followed in the CBA and AW mice. The spatula or curette method was employed to obtain a sample of the vaginal contents which were examined after staining with methylene blue (Snell, 1941). These mice were accustomed to frequent handling and appeared to be undisturbed by the daily weighing or spatulation.

Adrenalectomy, carried out in the manner previously described (p.40), was less difficult in mice of the AW and NZC strains than in CBA or albino mice. However, mice of the AW and CBA strains appeared to be more sensitive, than albino mice of equivalent age, to the deleterious effects of prolonged ether anaesthesia. This was reflected in an initially high proportion of animals dying while the operation was in progress, but not in an undue proportion of short survivals. Careful attention to the depth and duration of anaesthesia reduced the proportion of animals dying during the operation.

Results

The detailed results obtained in this experiment are tabulated in the appendix (Tables XVIII and XIX). The distribution of deaths and indefinite survivals is

summarised in Table VIII. Apparent differences in this table have been examined statistically by calculating the probability that the effect observed was due to chance, in the appropriate 2 x 2 contingency table (Goulden, 1952, pp. 372 et seq.)

The mean survival periods and body weight changes for those mice dying from the effects of adrenal insufficiency prior to the twenty-first post-operative day are shown in Table IX. Mean differences were appraised statistically by computing approximate "t" values for a number of individual comparisons (Cochran and Cox, 1950, pp. 91 et seq.). In view of the preliminary nature of the study, and because the numbers of animals involved were samall, it was considered that the application of more exact statistical techniques was not warranted.

Incidence of Post-Operative Shock and Indefinite Survival.

The incidence of post-operative shock, as evidenced by the occurrence of survival periods of less than 36 hours, was low in all three groups of mice (Table VIII). Indefinite survival was not recorded in the NZC or CBA mice. There was no evidence, therefore, that mice of these strains possessed functional accessory cortical tissue.

On the other hand, the number of indefinite survivars occurring in the small number of mice of the AW strain was relatively high (24 per cent of all mice operated). It appeared that a proportion of mice of this strain possessed accessory cortices capable of functioning at a level sufficienct for complete protection in a "neutral" environment. The occurrence of indefinite survival did not appear to be affected by the addition of sodium chloride to the drinking fluid. The greater proportion of survivors in male mice evident in Table VIII appeared to represent chance effects in sampling (probability approximately 8 per cent) rather than a real difference. It was tentatively concluded that a difference in the sex of the operated animal did not restrict the occurrence

of functional accessory cortices in mice of the AW strain.

Effect of Sex and Added Sodium Chloride upon Survival

NZC Mice. The addition of sodium chloride to the drinking water did not promote prolonged survival in mice of the NZC strain. The small mean difference between the two groups of male mice (Table IX) was not significant. It reflected the presence in the "saline group" of a single mouse which survived to the twelth post-operative day. Apart from this animal the distribution of deaths in the two groups appeared very similar.

CBA Mice. In the group of CBA mice one animal of each sex survived throughout the period of salt administration, but died within a few days of the substitution of tap water for the saline drinking fluid. The probability that this apparent effect of salt treatment was due to chance was approximately 20 per cent (Table VIII). However, the mean difference between the lifespan of salt-treated and control mice dying before the twenty-first day (Table IX) was significant at the 5 per cent level. It seemed, therefore, that the addition of sodium chloride to the drinking water of CBA mice alleviated adrenal insufficiency, at least in part, in a proportion of adrenalectomised animals.

No effect of sex upon the life-span of adrenalectomised CBA mice could be demonstrated.

AW Mice. The addition of sodium chloride to the drinking water appeared to have resulted in prolonged survival in a proportion of male AW mice. Female mice did not show a similar response (Table VIII). The probability that this sex-difference was due to chance occurrence in sampling was approximately 6 per cent. The probability that the difference in response of the two groups of males was due to chance was approximately 11 per cent. Neither null hypothesis could be rejected (or accepted) with confidence.

The mean survival periods for those animals of each sex on each treatment

that died prior to the twenty-first post-operative day are shown in Table IX. The difference between the two male treatment groups was not significant; those between the male and female mice receiving tap water and between the two female groups were significant at the 5 per cent level. These results emphasized the possible sex difference in response to sodium chloride administration revealed in Table VIII. It was concluded that sodium chloride alleviated adrenal insufficiency in male mice, but not in female mice of the AW strain, and that untreated female mice of this strain survived longer than untreated male mice. These conclusions were tempered by the realisation that the statistical procedures used to make multiple comparisons did not provide the same level of protection as when used in single comparisons.

The Effect of Ovarian Response upon Survival

An attempt was made to relate the response of the ovaries to adrenalectomy, as reflected in changes in the vaginal smear, to the life-span of adrenalectomised female mice of the CBA and AW strains. A number of types of ovarian response were postulated and mice were classified according to the supposed relation of type of smear to ovarian function (Snell, 1941). Subsequent experience with the vaginal smear technique indicated that these classifications were not satisfactory and thus the lack of any obvious relation between the supposed ovarian reaction (see Appendix, Table XIX) and survival time was not surprising.

Body Weight Changes

All mice (of the CBA and AW strains) that died prior to the twenty-first post-operative day lost body weight in period between adrenalectomy and death. The mean values, together with standard errors, for the various groups of mice are shown in Table X. These mean values were remarkably similar in the case of mice of the AW strain, while the small differences between male and female CBA mice were not significant.

All mice surviving beyond the twenty-first day maintained their body weights over the 21 day period; the majority gained in body weight. The mice that eventually died gained on average 1.66 gm in body weight during the treatment period whereas those animals which survived indefinitely showed a mean increase in body weight of 3.24 gm over the same period. This difference was significant at the 5 per cent level (by approximate "t" test).

Those mice that died after their saline drinking fluid was replaced by tap water lost body weight over the period between the twenty-first post-operative day and death. The mean body weight loss for the five mice in this category (3.52 gm) was similar to that obtaining from operation to death in mice dying before 21 days.

There was no consistent relationship between weight loss and survival period in mice dying before the twenty-first post-operative day. In the CBA mice the average within group correlation (Snedecor, 1945, pp. 325 et. sqq.) between survival period and body weight loss ($r = +0.581$) was significant at the 5 per cent level, but the individual within group correlations were non-homogeneous and ranged from 0.929 to -0.631. In the AW mice the within group correlations were consistently positive, but the average coefficient ($r = +0.301$) was not significantly different from zero.

Discussion

The experiment described in this section was designed primarily to provide preliminary information on the likely response of adrenalectomised mice of three strains to sodium chloride administration. The results obtained, therefore, do not warrant more than brief discussion.

Indefinite Survival

Evidence of the presence of accessory cortices, sufficiently active to sustain life, was provided by the occurrence of indefinite survival in adrenalectomised mice of the AW strain. In this respect mice of this strain appeared to resemble those of the strains operated by Dorfman, et al (1946) and Masui (1928). In contrast with the observations of Gaunt and his colleagues (Gaunt, Tobin and Gaunt, 1935) with adrenalectomised rats, the occurrence of functional accessories did appear to be greater in those animals in which survival was promoted by salt administration.

No cases of indefinite survival were detected in NZC or CBA mice after adrenalectomy. In so far as the very small numbers of mice operated constituted an adequate sample, mice of these strains appeared not to possess accessory cortices capable of readily assuming functional importance. In this respect CBA and NZC mice were similar to mice of the albino strain (see previous sections in this chapter), and to the strains of mice adrenalectomised by Hill (1948) and Howard (1946).

Lifespan After Adrenalectomy

The mean survival period of the untreated group of immature male NZC mice was very similar to that of albino mice of a similar age and sex. Mice of the AW strain appeared to survive slightly longer on average than similarly untreated albino mice. The survival period of the mice of the CBA strain approached that

reported by Hill (1948) with older mice of the related CHI strain.

There was little indication of amelioration of the effects of adrenal insufficiency, as a result of the addition of 1 per cent of sodium chloride to the drinking fluid in NZC mice. In both the CBA and AW strains of mice, however, it appeared that at least a proportion of mice were able to survive, when given saline to drink. In the AW mice this response appeared to be restricted to the male sex, and the pattern appeared to be similar to the "all or none" response reported by Gaunt, et al (1935) with Wistar rats, in that the adremalectomised mice appeared to respond by not dying until after the salt was discontinued, or to show no response at all to added salt. In the case of the CBA mice, although only one animal of each sex was enabled to survive throughout the treatment period, the mean lifespan of those mice which died during the period of salt administration was greater than that of mice given tap water to drink. The pattern of response of mice of this strain resembled that reported by Howard (1946) with immature mice of the H strain. Marder (1950) reported that approximately 80 per cent of adrenalectomised mature CBA mice survived while fed bread and milk and allowed to drink 1 per cent saline. The effects of the diet and drinking solution were, however, not investigated separately, nor was possible complication from the presence of functional accessory cortices excluded (Cf. ante, pp. 6 et seq.; p. 24). Bread was reputed to promote survival partly on account of its low potassium content (Cleghorn, et al., 1936) and Howard (1946) obtained prolonged survivals in adrenalectomised mice of the H strain when a high sodium intake was reinforced by a low potassium intake.

It seemed, therefore, that with more rigid control of the potassium content of the diet, than was obtained in the present investigation (Cf. ante, p. 61), mice of the CBA strain would be suited to the study of the effects of total adrenal insufficiency upon the mammary gland. The results of this preliminary investigation indicated that further studies with mice of the CBA strain were warranted.

Summary

The response of immature male mice of the NZC strain, and mature male and female mice of the CBA and AW strains to sodium chloride administration after adrenalectomy was studied. This treatment promoted the survival of a proportion of Aw male mice, partly alleviated the effects of adrenal insufficiency in male and female CBA mice, and had no apparent effect upon the survival of immature male NZC mice.

Indefinite survival, indicating the presence of functional accessory cortices, was observed only in mice of the AW strain.

The loss of body weight, which preceded death, was not affected by sex or by sodium chloride administration. There was no consistent relationship between survival period and body weight loss.

An unsuccessful attempt was made to relate the length of the post-adrenalectomy lifespan and the ovarian response to adrenalectomy.

CHAPTER IV

MAINTAINENCE OF LIFE IN IMMATURE ADRENALECTOMISED MALE ALBINO MICE WITH CORTISOL ACETATE.

There appeared to be only one published investigation of the level of adrenal steroids required to maintain life in adrenalectomised mice (Pfeiffer and Hooker, 1940), although this preparation has been used in bioassays of adrenal hormones involving actions other than life maintainence (Dorfman, 1954). The effect of adrenal steroids other than DCA (Pfeiffer and Hooker, 1940) upon the lifespan of the adrenalectomised mouse had not been the subject of any published investigation.

The study reported in this chapter was designed primarily to provide information on the lowest levels of cortisol acetate which would prevent death in the majority of adrenalectomised albino mice. Further, it was hoped by the substitution of this steroid for the endogenous adrenal secretion, to establish conclusively that the mortality observed in the albino strain of mice following adrenalectomy was the result of adrenal insuficiency.

It was considered desirable to make use of a steroid hormone known to be present in quantity in the endogenous adrenal product of the mouse. No information was available on the nature of this secretion, but evidence obtained with other rodents (Bush, 1953), suggested that corticosterone was probably quantitatively more important than cortisol. The latter compound was, however, the more readily available and was therefore used. Cortisol acetate was used in preference to the alcohol; firstly because it appeared that the former had a more prolonged period of action (Porter and Silber, 1953), and secondly because it was available in the more convenient form for parenteral administration.

Pfeiffer and Hooker (1940) reported that, at the level which protected adrenalectomised mice allowed to drink water, DCA caused a marked reduction in the lifespan of adrenalectomised mice given a two per cent sodium chloride solution to drink. It was decided, therefore, to investigate the effect of several levels of sodium chloride upon the survival of cortisol-treated adrenalectomised mice.

Materials and Methods

Animals

Immature male mice from the previously described albino colony were weaned at 21 days and adrenalectomised at 23 days from birth. The total of 60 mice included in this investigation comprised forty-eight that were bilaterally adrenalectomised and twelve that had the right adrenal only removed. The capsules of all glands removed in the course of bilateral adrenalectomy were carefully examined. Those animals, in which the integrity of either capsule was uncertain, were not included in the experiment.

Application of Treatments

Animals were allocated at random, within each of four replications, to one of the several treatments. In each block twelve bilaterally adrenalectomised mice were treated, according to a factorial design, with one each of four levels of cortisol and three levels of sodium chloride. The three unilaterally operated animals in each block received one each of the three levels of sodium chloride; these mice were not injected with adrenal steroid.

Cortisol acetate was injected subcutaneously in daily doses of 12.5 ug., 25 ug., 50 ug., and 75 ug. These levels were selected, on the results of several pilot trials, to include within their range the minimal level of the hormone which would maintain the majority of adrenalectomised mice and promote a "normal" growth rate over a three week treatment period. Each dose was given in 0.05 ml. of diluent. The stock solution for each dose level was prepared by adding the requisite volume of physiological salt solution (0.9% w/v sodium chloride) to an Upjohn suspension* of cortisol acetate. The several constituents of this diluent

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* Cortef Acetate Suspension (Lot No. 1148; Batch No. 6): cortisol acetate, 25 mg./ml.; polysorbate 80 U.S.P., 2 mg./ml.; carboxymethylcellulose (sodium), 2.5 mg./ml.; benzyl alcohol; 0.9% w/v physiological salt solution, q.s.

had previously been found by Flux (1953) to be without effect on the mammary glands, adrenals or ovaries of mice, or upon the response of pregnant and lactating rats to adrenalectomy. The effect of the diluent was not checked in the present investigation.

Sodium chloride was administered in the drinking water. The three drinking fluids were: tap water; tap water plus 1 per cent (w/v) sodium chloride; tap water plus 2 per cent (w/v) sodium chloride.

Treatment was commenced on the day of operation and continued for twenty-one days.

Diet, Post-Operative Conditions, Recording

The feed described in the previous chapter was given ad libitum. Mice were kept before and after the operation in controlled temperature conditions, similar to those described in the previous chapter.

Body weights were recorded at three day intervals during the treatment period and thereafter till death or until indefinite survival became certain. Additional body weights were taken over the period when mice showed symptoms of adrenal insufficiency. This procedure ensured, as far as possible, that live-weights were obtained for all mice within the 24 hours that preceded death.

All animals that survived to slaughter (30 days after the end of the treatment period) were examined for accessory cortices. Macroscopic inspection of the anterior portion of the abdominal cavity, together with histological examination of any suspicious tissues, failed to reveal the presence of accessory adrenal tissue in any indefinite survivor.

TABLES XI, XII, XIII.

TABLE XI

SURVIVAL, AND BODY WEIGHT CHANGES IN CORTISOL-TREATED MALE ALBINO MICE ADRENALECTOMISED AT TWENTY ONE DAYS OF AGE: SUBCLASS NUMBERS AND MEANS

DRINKING SOLUTION:	TAPWATER WITHOUT ADDED SODIUM CHLORIDE					TAPWATER + 1 PER CENT SODIUM CHLORIDE					TAPWATER + 2 PER CENT SODIUM CHLORIDE				
TYPE OF OPERATION:	BILATERAL ADRENALECTOMY				CONTROL	BILATERAL ADRENALECTOMY				CONTROL	BILATERAL ADRENALECTOMY				CONTROL
LEVEL OF CORTISOL (ug./day):	12.5	25.0	50.0	75.0	-	12.5	25.0	50.0	75.0	-	12.5	25.0	50.0	75.0	-
						NUMBER OF MICE									
DYING DURING PERIOD OF TREATMENT	1	3	-	-	-	2	1	-	-	-	-	-	1	-	-
DYING AFTER TREATMENT CEASED	2	1	2	2	-	2	3	1	2	-	3	3	2	3	-
SURVIVING INDEFINITELY	1	-	2	2	4	-	-	3	2	4	1	1	1	1	4
						SURVIVAL PERIOD (days)									
ALL MICE DYING*	19.67	14.75	25.50	29.00	-	18.00	21.25	24.00	29.00	-	25.67	24.33	23.33	25.67	-
DYING AFTER TREATMENT CEASED**	5.00	6.00	4.50	8.00	-	3.50	3.67	3.00	8.00	-	4.67	3.33	5.50	4.67	-
						GAIN IN BODY WEIGHT DURING PERIOD OF TREATMENT (gms)									
DYING DURING PERIOD OF TREATMENT**	-1.60	-1.23	-	-	-	-0.50	-1.30	-	-	-	-	-	-0.20	-	-
DYING AFTER TREATMENT CEASED	4.05	3.20	5.35	6.05	-	3.60	4.43	3.80	3.95	-	2.93	4.50	3.65	4.23	-
SURVIVING INDEFINITELY	7.70	-	6.30	5.50	9.15	-	-	8.13	4.35	8.20	2.50	3.40	5.10	4.85	8.35

TABLE XII

EFFECT OF CORTISOL ACETATE AND SODIUM CHLORIDE ON THE INCIDENCE OF INDEFINITE SURVIVAL

	LEVEL OF CORTISOL ACETATE (ug./day)								
	12.5	25.0	Total	50.0	75.0	Total	12.5 & 25	50 & 75	Total
Dying	10	11	21	6	7	13	21	13	34
Not Dying	2	1	3	6	5	11	3	11	14
Total	12	12	24	12	12	24	24	24	48
							p = 0.013		
	LEVEL OF ADDED SODIUM CHLORIDE (%)								
	0	1	Total	0	2	Total	1	2	Total
Dying	11	11	22	11	12	23	11	12	23
Not Dying	5	5	10	5	4	9	5	4	9
Total	16	16	32	16	16	32	16	16	32

*Lifespan from time of adrenalectomy **Lifespan after treatment concluded

*
**Loss in body weight over the period from adrenalectomy till death

**Excluding animals which did not die upon cessation of treatment

TABLE XIII

SURVIVAL, AND BODY WEIGHT CHANGES IN CORTISOL-TREATED MALE ALBINO MICE ADRENALECTOMISED AT TWENTY ONE DAYS OF AGE: WEIGHTED MEANS AND THE RESULTS OF STATISTICAL TESTS OF SIGNIFICANCE

	SURVIVAL PERIODS				BODY WEIGHT GAIN
	ALL MICE DYING		DYING AFTER TREATMENT		MICE ALIVE AT 21 DAYS
	days*	log.* days	days**	log.** days	gm
CORTISOL ACETATE (ug./day)					
12.5	20.80	1.280	4.43	0.637	3.44 ** *
25.0	19.73	1.262	3.86	0.562	4.29
50.0	24.17	1.378	4.60	0.645	4.36
75.0	27.57	1.439	6.57	0.797	4.72
SODIUM CHLORIDE (% added)					
0	20.64	1.265	5.86	0.748	4.87** *
1	21.73	1.311	4.62	0.626	4.02
2	24.75	1.391	4.45	0.632	3.84
GENERAL MEAN	22.44		4.88		4.17
DEGREE OF ADRENALECTOMY	(Means joined by a vertical line did not differ significantly: p > 0.05 by "F" test plus a range test or by approximate "t" test)				
Bilateral, dying					4.17
Bilateral, surviving					7.89
Unilateral (control)					8.57

Results

The numbers of mice dying or surviving, the mean survival periods and the mean body weight changes for the individual treatments are shown in Table XI. The results of statistical examination of the proportions of animals dying or surviving are shown in Table XII, and the weighted means, together with the results of statistical tests, for each of the levels of cortisol acetate and sodium chloride are presented in Table XIII. The analyses of variance of these means are summarised in Table XXI.

Indefinite Survival

Following the period of treatment with cortisol acetate a proportion of adrenalectomised albino mice survived indefinitely. It seemed that the delayed onset of adrenal insufficiency, brought about by the administration of adrenal steroid, allowed accessory cortices to hypertrophy sufficiently to support life in a proportion of adrenalectomised male mice.

The number of indefinite survivors appeared to be greater in those groups of mice treated with 50 and 75 ug. cortisol acetate than in those injected at the two lower levels. This apparent difference was examined statistically by estimating (Goulden, 1952, pp. 372 et. seq.) the probability that it was due to chance (Table XII). The estimate obtained ($p \doteq 0.01$) indicated that this could represent a real difference. The proportion of indefinite survivors did not, however, appear to be affected by the level of sodium chloride in the drinking fluid.

Survival Periods of Mice Dying

The effect of the treatments upon survival was examined by an analysis of variance of the survival periods of all mice dying, and by a separate analysis

of the post-treatment lifespan of mice that died after the period of cortisol administration terminated. These analyses of variance (Table XXI) were carried out after the data had been transformed to a logarithmic scale, on which the relationship between subclass means and variances appeared to be minimised. In each analysis the subclass numbers were unequal, but since these inequalities were the result of the various treatments they were assumed to be representative of population proportions and analysed accordingly (Snedecor, 1943, pp. 284 et seq.) The validity of this assumption of proportional subclass numbers was of no importance in the analysis of the survival periods of all mice dying, where the analysis was not carried beyond the stage of testing the significance of differences between the individual treatment means.

There were no significant differences between the individual treatment means for the survival periods of all mice dying. However, in the case of the post-treatment lifespan the subclass means were significantly different. Further "F" tests were constructed and these indicated that there were significant differences between the weighted means for cortisol acetate levels (Table XIII). There were no significant differences between the weighted means for levels of added sodium chloride (Table XIII), and the interaction between levels of cortisol and levels of sodium chloride was not significant. The multiple range test of Duncan (1955) was used to detect the whereabouts of significant differences between the means for the levels of cortisol acetate. This test revealed that the post-treatment lifespan of mice receiving 75 ug. of hormone was significantly greater than that of the mice receiving 50 ug. The post-treatment survival periods of mice treated with 50, 25 and 12.5 ug. of cortisol did not differ significantly.

Body Weight Changes

The subclass means for body weight changes during the treatment period (or between the operation and death in the case of those mice dying during the treatment period) are shown separately, in Table XI, for mice dying during the treatment period, for mice dying after the treatment period, and for mice surviving indefinitely.

All animals dying during the period of cortisol administration showed a loss of body weight, which appeared to commence immediately post-operatively and continue till death. The numbers of animals involved were too small to allow even the most cursory statistical analysis.

The body weight gain of mice dying after the discontinuation of treatment was subjected to analysis of variance (Table XX). The differences between the subclass means were not significant. A log-dose-response relationship between level of cortisol administration and body weight gain was suggested by the means for each of the four levels of hormone (Table XIII). However, an analysis of covariance did not reveal significant components due either to regression, or to deviations from the regression (Table XXI). In view of the small numbers of animals involved, it seemed that the failure to demonstrate significant "F" values, in either of the analysis of variance or covariance, may have reflected the low precision of the estimates of the residual mean squares, rather than the absence of a real effect of level of cortisol acetate administration upon body weight gain.

The significance of the differences in body weight gain during the treatment period between control-operated mice, bilaterally adrenalectomised mice that survived indefinitely, and totally adrenalectomised mice that died after the discontinuation of hormone administration, was examined using an approximate "t" test (Cochran and Cox, 1950, pp. 91 et seq.). The mean body weight increase for the mice dying was significantly less than those for control operated or indefinitely surviving adrenalectomised mice. The mean body weight gains of the latter two groups of mice did not differ significantly.

Discussion

Indefinite Survival, Accessory Cortical Tissue

The results obtained in the present experiment contrasted with those described for albino mice in Chapter III. In the previous experiments all totally adrenalectomised mice of this strain died; in the present experiment a proportion of immature male mice survived indefinitely after the conclusion of a 21 day period of cortisol acetate injection. A similar effect of cortin administration--the disclosure of unsuspected accessories--was reported by Gaunt and Gaunt (1934) in adrenalectomised rats of Wistar origin. The delayed onset of the terminal stages of adrenal insufficiency appeared, in these two groups of animals, to allow hypertrophy of accessory cortical tissue to an extent that, in a proportion of animals, it would sustain life. The failure of the majority of adrenalectomised mice to survive indefinitely, after the discontinuation of the exogenous supply of cortical hormone, did not imply that these mice lacked accessory cortices. If these were present in mice that died then they did not hypertrophy sufficiently to support life.

The greater incidence of indefinite survival in the groups of mice receiving the two higher levels of cortisol acetate than in the groups treated with the two lower levels of hormone appeared to be at variance with the reported (Ingle and Higgins, 1938; and others) effects of corticoid administration upon the regeneration of enucleated adrenals in rats. Exogenous adrenal hormones depressed the rate of regeneration, but this effect--mediated presumably by repression of ACTH release--was not evident at doses below a certain level. It was conceivable that the blood levels of cortisol produced in the present experiment were too low to affect the manifold increase in ACTH release which has been shown to occur after adrenal removal (Gemzell, et al., 1951; Sydnor

and Sayers, 1952. However, the injection of similar doses of cortisol acetate in overiectomised mice produced significant reductions in adrenal weight (Cf. post, Table XIV).

An effect of cortisol administration upon ACTH release in the adrenalectomised preparation could be reconciled with the increased incidence of functional accessories in mice treated with the higher levels of cortisol, provided certain assumptions were made regarding the thesholds for effects upon life maintenance and inhibition of ACTH release. The assumptions required were: that the two thresholds were correlated, and that the threshold for ACTH inhibition was higher than that for life maintenance. At a given level of cortisol administration, adrenalectomised mice could be arranged in three classes, ordered according to decreasing sensitivity to exogenous cortisol: mice (surviving only as long as treatment continued) where the level of exogenous cortisol exceeded both thresholds; mice (surviving indefinitely, provided they possess accessory cortices capable of hypertrophy) where the level was between the two thresholds; mice (dying during treatment) where the level was below both thresholds. At dose levels above or below such a given level the numbers of mice in each class would be expected to differ. The exact numbers in each class would depend upon the relation of the dose levels to the average difference between the supposed thresholds, but a distribution similar to that found in the present investigation could easily be obtained.

It has not been necessary to postulate a lack of accessory cortical tissue in any group of animals. However, were such tissue absent in a number of mice the hypothesis would not be upset. The animals lacking accessory tissue would fall into one of the two extreme classes. The validity of the assumptions could only be determined by further investigation employing a wider range of dosage levels, greater numbers of mice at each dose level, and including the measurement of the

level of ACTH release.

The Level of Cortisol Acetate Required for Life-Maintenance

At each of the four levels of steroid administration, the majority of cortisol-treated adrenalectomised mice were able to survive and, over the 21 day treatment period, grew in body weight at a rate approximately half that of control-operated animals.

The interpretation of the relation of body weight changes to degree of adrenal insufficiency in adrenalectomised animals treated with 11-oxygenated adrenal steroids is complicated by the inhibition of growth which results, per se, from the administration of these steroids. Ingle (1940, 1950) reported that, although they alleviated other symptoms of adrenal insufficiency, 11-oxygenated adrenal steroids caused a loss in body weight in adrenalectomised rats; this loss was of a greater order than that produced in intact rats by similar treatment.

The three lower doses of cortisol acetate employed in the present investigation when administered for a similar period, caused significant reductions in the rate of body weight gain of ovariectomised immature mice (Cf post, Table XIV). These levels, however, did not appear to have adversely affected the body weight increase of adrenalectomised mice that possessed accessory cortices capable, at the end of the injection period, of maintaining life. The body weight gains of these mice was only slightly, and not significantly, less than that of control-operated mice. It appeared, therefore, that the failure of cortisol-treated adrenalectomised mice to grow in body weight at a rate comparable with that of control-operated mice, or cortisol-treated mice possessing accessories was due, at least in part, to sub-optimal levels of corticoid administration.

No significant differences were demonstrated between the effects of the

several levels of cortisol upon survival time (for all mice dying), or upon body weight gain (in mice dying after the end of treatment). In view of the small numbers of mice employed and the lack of any statistical control of within-group variability, the possibility that the positive relation between dosage level and survival period or body weight gain seen in this sample of mice represented a real graded response to level of cortisol administration could not be neglected.

In the only published account of the maintenance of adrenalectomised mice with adrenal steroids, Pfeiffer and Hooker (1940) reported that the daily administration of 250 ug. DCA (in 0.05 ml. sesame oil) would maintain sexually mature adrenalectomised mice of the A, C_3H, and NH strains, and promote a "normal" increase in body weight. Desoxycorticosterone and its derivatives have generally been found more effective per unit weight, as regards promotion of survival, than the 11-oxygenated steroids (Cf ante, pp. 26a et seq.), so that the reported requirement with mice of the three inbred strains seemed several times greater than that found with immature albino mice in the present study. The relation of the level of DCA administration to the minimal requirement of the inbred strains of mice was not clear, since Pfeiffer and Hooker (1940) did not report the effectiveness of other levels of DCA. When drinking water of adrenalectomised mice injected with 250 ug. DCA was replaced by a 2 per cent solution of sodium chloride toxic reactions were observed (Pfeiffer and Hooker, 1940). This would suggest that the minimal level of DCA required for life maintenance was somewhat less than that reported by Pfeiffer and Hooker for these inbred mice. Adverse effects of DCA, in the presence of an increased intake of sodium salts, have been obtained in other species only with what were described as "high" levels of the hormone (Kuhlman, Ragan, Ferrebee, Atchley and Loeb, 1939; Goodof and MacBryde, 1944).

An increase in body weight of adrenalectomised mice during treatment with DCA (Pfeiffer and Hooker, 1940) of an order similar to that obtaining in intact control mice was not necessarily indicative of normal growth in body weight. At moderate dose levels DCA causes a considerable retention of water (see Noble, 1950, 1955) and what appeared to be gains in amount of body substance may have been simply the result of increased hydration of the tissues.

The Effect of Sodium Chloride Administration

In the present study the level of sodium chloride in the drinking fluid did not appear to have affected either the lifespan or the body weight gain of cortisol-treated adrenalectomised mice. There did not appear to be any reports relating to the effect of sodium level upon the life-maintaining potency of 11-oxygenated steroids, butthere were several reported observations of the effect of dietary level of sodium salts upon the life-maintaining action of DOC and DCA.

The toxic effects of high levels of DCA were emphasized in the presence of an increased intake of sodium (Kuhlman, et al., 1939; Goodof and MacBryde, 1944). When the dietary level of sodium was low the life-maintaining potency of DCA was reduced (Eversole, 1945a; Cleghorn, Fowler, Wenzel and Clarke, 1941).

Post-Treatment Lifespan

The mice injected with 75 ug. of cortisol acetate daily survived on average for a significantly longer period after cessation of treatment than did mice injected with any of the three lower doses of this steroid. This difference in post-treatment lifespan could have resulted from a disparity in the duration of effective therapy, arising from differences in the period for which the subintaneous reservoir of injected steroid persisted. Alternatively, the difference may have reflected a more nearly normal state of adrenal sufficiency in the mice injected with the highest level of cortisol acetate. Regardless of the

relation of the effect to either or both of these possible underlying causes, the similarity of the lifespans for the three lower doses and the marked difference between these and that for the highest dose suggested a threshold response. However, it seemed probable that the threshold between the two higher levels of cortisol was more apparent than real, particularly since increasing doses of cortisol acetate appeared to evoke a graded response in respect of body weight gain. In any event the small number of mice involved, and the consequent low precision of the estimates of residual variance precluded definite conclusions regarding the nature of either the survival or growth response.

The post-treatment mean survival periods for mice receiving each level of cortisol were greater than the post-operative lifespan reported (see Table V) for male mice adrenalectomised at a similar age, but not treated with cortisol acetate. This difference was not surprising in view of the greater age (42 days) at the time of onset of "total" adrenal insufficiency in the cortisol-treated mice. The post-treatment mean lifespan for male albino mice adrenalectomised at 21 days of age and treated with any of the four levels of cortisol for three weeks (4.88 days) was very similar to the post-operative mean survival period for mice of similar strain and sex adrenalectomised at 42 days of age (4.85 days). Of the four groups of cortisol-treated mice only that injected with the highest level of the adrenal steroid had a greater mean lifespan that that of the group of similar mice exposed to adrenal insufficiency at an equivalent age; the means for the four groups of cortisol-treated mice (Table X) were all within the range of "block means" for untreated mice adrenalectomised at 45 days of age (Table VI).

Firor and Grollman (1933) suggested that the total effect of operative procedure could be evaluated by comparing the survival times of two groups of

adrenalectomised animals, where one group had received a short period of "efficacious therapeutic treatment". On the basis of the above comparisons then it could be concluded that the mean survival periods for mice adrenalectomised at 45 days of age (shown in Tables V and VI) obtained in an earlier investigation did not include effects due to post-operative shock. The result of these comparisons then tended to confirm the hypothesis (Cf. ante, pp. 46 et seq.), at least in the case of mice adrenalectomised at 42 days of age, that the exclusion of data pertaining to mice dying within 36 hours of operation eliminated the effect of post-operative shock. This confirmation would have been more emphatic had it been based upon a comparison of mice operated at the same time.

Summary

The majority of male mice adrenalectomised at 21 days from birth were able to survive, and grow in body weight at about half the rate of control-operated mice as long as treatment with cortisol acetate in daily doses ranging from 12.5 to 75 ug. was continued. These levels appeared to be slightly sub-optimal. A proportion of the mice were enabled to survive indefinitely after the 21 day injection period ended. These mice, which presumably developed functional accessory cortices, grew at a rate only slightly less than that of control-operated mice. The number of indefinite survivors was greater at the two higher levels of cortisol treatment than at the two lower levels.

The average lifespan appeared to be greater and the number of short survivals (less than 21 days) appeared to be less at higher levels of cortisol administration, but these differences were not significant.

All mice dying showed a loss in body weight during the terminal stages of adrenal insufficiency. Those mice dying after the injection of hormone ceased gained in body weight during the treatment period. A positive linear log. dose-response relationship was suggested, but proved to be non-significant. The mice which survived indefinitely gained in body weight over the injection period at a rate similar to that of control-operated mice, and greater than that of mice dying after conclusion of treatment.

Confirmation of the hypothesis regarding the influence of post-operative shock advanced in Chapter III was obtained by comparison of the duration of survival after the imposition of adrenal insufficiency in cortisol-treated and untreated mice.

CHAPTER V

THE EFFECT OF CORTISOL ACETATE ON OESTRONE-INDUCED MAMMARY GLAND GROWTH IN IMMATURE OVARIECTOMISED ALBINO MICE

The effects of 11-oxygenated adrenal steroids upon mammary development have been investigated in the rat and in the mouse. Whereas in the rat it appeared that cortisol and cortisone might stimulate mammary gland growth (Johnson and Meites, 1955; Selye, 1954a,b), the results obtained by Flux (1953, 1954) indicated clearly that 11-oxygenated adrenal steroids in moderate doses inhibited oestrone-induced mammary development in ovariectomised immature CHI mice.

Death could be prevented in immature adrenalectomised male albino mice (see Chapter IV) by injection of cortisol acetate in doses of a lower order than those employed by Flux (1953, 1954) in mammary growth studies. It was of interest to determine whether these lower levels of cortisol would affect oestrone-induced mammary gland growth.

Materials and Methods

Animals

Immature female mice of the previously described albino strain, ovariectomised at weaning (21 days of age), were used in the investigation. The technique of ovariectomy employed was that described in a previous chapter (pp. 50 et seq.). Six intact animals were included as controls.

Application of Treatments

Ovariectomised mice were treated according to a factorial design incorporating three levels of oestrone (zero, 0.003 ug., and 0.006 ug. daily) and four levels of cortisol acetate (zero, 12.5 ug., 25 ug., and 50 ug. daily) in all possible combinations. Mice were allocated at random to the thirteen

individual treatment groups (which included the intact control group) in each of six replicates. Treatment commenced on the day of operation and continued for 21 days. This treatment period was selected on the basis of results obtained with mice of the albino strain (Flux, unpublished). In these mice the mammae responded to stimulation at 21 days of age, and by 42 days the differences between the mammary glands of intact and ovariectomised mice were sufficiently marked to permit detection of the effect of stimulation in ovariectomised mice. The doses of oestrone were chosen to provide two distinct levels of mammary stimulation, which could be distinguished from the degree of development exhibited in untreated intact and ovariectomised mice. The differences were sufficient to permit recognition and measurement of any inhibition or stimulation resulting from cortisol acetate administration. The levels of cortisol acetate used approximate the minimal daily requirement for the maintenance of life in immature adrenalectomised male mice (see Chapter IV).

Oestrone was administered subcutaneously in arachis oil by means of an "Agla" micro-syringe, which permitted the injection of the required doses in small volumes (0.003 and 0.006 ml.) and avoided the accumulation of oil at the injection site. Cortisol acetate was injected subcutaneously as a microcrystalline suspension in 0.05 ml. of an acqueous medium, which has been described in Chapter IV (see footnote p.77). The two steroids were injected at separate sites.

Preparation and Measurement of Mammary Glands

Whole mounts of all the glands of each mouse were prepared by the method described by Flux (1953), which was adapted from that described by Cowie (1947) and Cowie and Folley (1947) for use with the rat. The techniques used to measure the extent and degree of branching of the duct systems were those described by Flux (1953).

The estimate of extent of the duct system was obtained for each mouse by measuring the total area covered by the ducts of all glands, whereas the estimate of arborescence depended upon the number of duct junctions in the first two pairs of thoracic glands. The restriction of the latter estimate was dictated partly by the difficulties involved in obtaining accurate and dependable values in the inguinal glands where connective tissue, which could not be removed without damaging the gland, often obscured the central portion of the duct system. The exclusion of the third pair of thoracic glands was suggested by Flux (1953), and followed in the present study, because the initial development of these glands was very variable and not related to the growth of the other glands. It was considered that an estimate based solely upon the first two pairs of thoracic glands was likely to be more closely related to that which would have been obtained if the number of duct junctions were counted in all glands, than one that included the third thoracic pair of glands.

Gland, Organ and Body Weights, Vaginal Smears

In order to provide concommitant information on the biological activity of the steroids at the doses levels used, the thymus and adrenal glands, and the uteri were removed and weighed at the conclusion of the experiment. Body weights were recorded immediately after ovariectomy and at intervals during the treatment period; dead body weights were determined at autopsy. As a further check on the activity of oestrone at the levels used, the times of vaginal introitus were recorded, and the nature of changes in the vaginal smear followed after introitus in ovariectomised mice. The time of vaginal opening and the changes in the vaginal smear were also recorded in the six intact control mice. The technique used, and the criteria employed to evaluate changes in the vaginal smear have been described in a previous chapter (p. 68).

Diet, Housing, Temperature Control

The diet described in Chapter III was fed ad libitum and all mice were allowed to drink tap water at will. All animals were housed in a room in which the temperature was controlled between 65° and 75°F. Mice were caged according to the level of oestrone administration, which avoided possible contamination of untreated animals with faecal or urinary estrogens.

Statistical Analysis

The data from one ovariectomised mouse, treated with 12.5 ug. cortisol acetate and 0.003 ug. oestrone were excluded and values calculated by missing plot analyses (Cochran and Cox, 1955, pp. 96 et seq.) substituted. The basis for the rejection of these observations was the demonstration that the uterus weight for this animal, by reason of its greater magnitude, was inconsistent with those for other ovariectomised mice treated with the same level of oestrone (Bliss and Calhoun, 1954, p.70). That this mouse was the only animal to show persistent oestrus after vaginal introitus was also indicative of some abnormality, and although ovarian remnants were not detected at autopsy it seemed that this animal possessed some endogenous source of oestrogen.

The analyses of variance of the three measures used to describe effects of the several treatments upon the mammae were carried out after transformation by taking the logarithms of the original observations. On purely theoretical grounds it was clearly impossible that more than two of these measures were normally distributed, on either the original or the transformed scale, since one of the three variates was the ratio of the other two.* The logarithmic metameter was selected, however, using a purely empirical criterion: the

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* This was not strictly correct since the measure of total area related to all the mammary glands of each mouse while the total duct junctions and duct junctions per unit area were estimated from the first two pairs of glands.

removal of a tendency for the subclass means and variances to be correlated. Although in each case this transformation was the most efficient of those tried it was not equally effective for all three variates. Taking logarithms appeared to "over-transform" slightly in the case of number of duct junctions per unit area, and "under-transform" in the case of area and total number of duct junctions.

The analyses of variance of the data obtained in body weights, adrenal and thymus gland weights, and uterus weights were carried out with the original data. The form of the analyses of variance (Snedecor, 1946, pp. 304 et seq.) for these and the mammary gland variables differed only in the extent to which the various treatment sums of squares were partitioned to allow the construction of "F" tests with single degrees of freedom.

Differences between ovariectomised mice and the intact untreated mice were evaluated by approximate "t" tests (Cochran and Cox, 1950, pp. 91, et seq.), as were differences in the time required for vaginal introitus. The differerences in the numbers of mice showing vaginal opening were examined by calculating the probabilities, in a series of 2 x 2 contingency tables, that these were due to chance, (Goulden, 1952, pp. 372 et seq.).

TABLE XIV

TABLE XIV

THE EFFECT OF THE ADMINISTRATION OF CORTISOL ACETATE AND OESTRONE UPON THE MAMMARY GLANDS AND OTHER ORGANS OF OVARIECTOMISED ALBINO MICE

(Except where otherwise indicated each group contains six mice. Operations performed at 21 days of age and treatments applied for 21 days.)

	Mammary Gland						Mean Uterus Weight	Mean Thymus Weight	Mean Adrenal Weight	Mean Initial Body Weight	Mean Gain in Body Weight	Vaginal Opening	
	Mean Total Area		No. of Duct Junctions (1st and 2nd pairs)									Number Reacting	Mean Time to Respond
	$mm.^2$	$\log_{10} mm.^2$	Total	$\log_{10}$ total	per $mm.^2$	$\log_{10}$ per $mm.^2$	mg.	mg.	mg.	g.	g.		days
Ovariectomised Controls	49.8	1.676	75.5	1.862	4.46	0.638	5.00	71.2	6.08	8.43	8.33	0	-
Ovariectomised and Daily Dose of:													
Cortisol acetate, 12.5 ug.	75.3	1.809	124.7	2.065	4.27	0.600	6.17	44.2	6.00	10.68	6.12	0	-
Cortisol acetate, 25.0 ug.	63.2	1.762	101.7	1.986	4.42	0.628	6.00	45.2	5.33	9.87	6.22	0	-
Cortisol acetate, 50.0 ug.	57.6	1.736	90.0	1.950	4.44	0.613	5.08	41.0	5.67	10.27	4.97	0	-
Oestrone, 0.003 ug.	86.0	1.926	137.7	2.114	3.59	0.528	6.42	59.6	6.25	10.13	7.57	3	17.7
+ Cortisol acetate, 12.5 ug.[1]	104.3	1.993	168.3	2.232	3.66	0.547	7.25	54.8	5.75	9.85	6.25	3	18.0
+ Cortisol acetate, 25.0 ug.	95.5	1.969	124.0	2.089	3.60	0.543	6.33	47.9	5.42	10.41	6.25	4	15.0
+ Cortisol acetate, 50.0 ug.	81.3	1.893	145.2	2.142	3.78	0.572	6.17	30.2	5.50	10.90	5.12	6	12.2
Oestrone, 0.006 ug.	169.9	2.186	231.5	2.344	2.94	0.462	7.67	56.9	6.33	10.48	7.62	6	13.3
+ Cortisol acetate, 12.5 ug.	176.7	2.374	276.2	2.434	3.38	0.504	8.42	44.8	5.67	10.28	5.90	6	13.2
+ Cortisol acetate, 25.0 ug.	163.4	2.197	203.8	2.295	3.02	0.467	8.17	45.8	5.17	9.62	6.50	6	13.5
+ Cortisol acetate, 50.0 ug.	125.9	2.205	191.0	2.244	3.85	0.579	8.67	35.7	5.25	11.47	4.37	6	12.0
Intact Controls	445.8		756.8		4.26		41.00	57.0	5.85	10.15	7.52	6	12.7
Significance of Differences													
Between Oestrone Levels[2]		**		**		**Blocks	**	*Cortisol	N.S.	N.S.	N.S.	-[4]	-
Treated v. Untreated		**		**		x Oestrone	**	x Oestrone	-	-	-	**[4]	-
Dose 0.003 ug. v. 0.006 ug.		**		+		Interact.	**	Interact.	-	-	-	**[4]	*[3]
Between Cortisol Levels[2]		+		*		N.S.	N.S.	**	**	N.S.	**	N.S.[4]	-
Treated v. Untreated		N.S.		N.S.		-	-	**	**	-	**	-	-
(Dose 12.5 ug. v. 25.0 ug.		-		-		-	-	N.S.	N.S.	-	N.S.	-	-
(Dose 50.0 ug. v. 25.0 + 12.5 ug.		-		-		-	-	*	N.S.	-	**	-	-
or													
(Dose 12.5 ug. v. 25.0 + 50.0 ug.		*		**		-	-	-	-	-	-	-	-
(Dose 50.0 ug. v. 25.0 ug.		N.S.		N.S.		-	-	-	-	-	-	-	-
Intact Controls v. Treated Groups[3]													

Mammary Gland Area : Greater** than mean for mice treated with 0.006 ug. oestrone per day.

Total Duct Junctions: Greater** than mean for mice treated with 0.006 ug. oestrone per day.

Duct Junctions/$mm.^2$: Not significantly from means for mice treated with either level of oestrone or from mean for mice not receiving oestrone.

Uterus Wt.: Greater* than mean for mice treated 0.006 ug. Oestrone

Thymus Wt.: Less* than mean for spayed controls, greater** than mean for mice treated 50 ug. cortisol daily.

Adrenal Wt.: Less* than mean for mice not treated with cortisol, greater+ than mean for cortisol-treated mice.

Initial Body Wt.: No significant differences.

Gain in Body Wt.: Not different(N.S.) from mean for mice not treated cortisol, greater** than mean for mice treated two lower doses of cortisol.

Time to Opening of Vagina: Not different(N.S.) from mean for mice receiving 0.006 ug. oestrone daily.

1. Data from one animal excluded and values calculated in a missing plot analysis substituted.
2. Significance of differences evaluated by the "F" test.
3. Significance of differences examined by approximate "t" test on untransformed data.
4. Significance of differences determined by calculating the probabilities for a series of 2X2 contingency tables.

**Highly significant ($p < 0.01$)
* Significant ($p > 0.01, < 0.05$)
+ Not significant ($p > 0.05, < 0.1$)
N.S. Not significant ($p > 0.1$)

Results

The results are shown in detail in Table XXII and are summarised, together with the outcome of statistical tests, in Table XIV. The detailed analyses of variance are presented in Tables XXIII and XXIV.

Body Weights

There were no significant differences between the initial body weights of the groups of mice allotted to the various treatments.

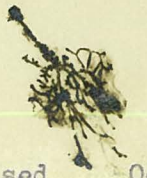
Ovariectomy and treatment with oestrone did not have significant effects on the rate of body growth during the injection period. Treatment with cortisol acetate, however, led to a significantly reduced gain in body weight over this period, which was most marked in the group of mice injected with the highest dose (50 ug./day). The growth of the two groups of mice treated with the lower levels of cortisol were very similar; these animals grew at a faster rate than those treated with the highest level of cortisol, but gained significantly less weight than intact control mice.

Mammary Glands

Photographs of mammary glands from a number of mice are shown in Figures 5 to 8. The whole mounts of second thoracic glands illustrated in Figures 5 and 6 exemplify the effects of oestrone and cortisol administration respectively. An example of the bilateral asymmetry characteristic of the third pair of thoracic mammary glands is illustrated in Figure 7, while Figure 8 serves to illustrate the variation between similarly treated mice and between the individual mammary glands of a single mouse.

In order that the response of the mammae might be examined independently of the general effect of cortisol acetate upon body size, an analysis of covariance, with the body weight at slaughter as the independent variable, was carried out for

Ovariectomised Control



Ovariectomised Oestrone 0.003ug.

Intact Control

Ovariectomised Oestrone 0.006ug.

FIGURE 5. SECOND THORACIC MAMMARY GLANDS FROM OVARIECTOMISED, INTACT AND OESTRONE-TREATED OVARIECTOMISED ALBINO MICE (Magnification 5.25 X linear).

(The levels of oestrone indicated in the figure were the daily doses.)

the estimate of total mammary area. The regression of mammary gland area on body weight at slaughter was not significant ($p > 0.10$), and accordingly the "F" tests of significance were carried out on the unadjusted treatment means (Table XIV).

The response of the mammary glands to the two doses of oestrone was similar to that anticipated when these were chosen. Significant mean differences in the extent and number of junctions of the duct systems of the mammae were demonstrated between oestrone-treated and oestrone-untreated ovariectomised mice, and between intact control mice and ovariectomised mice treated with the higher dose (0.006 ug./day) of oestrone. The differences between the areas covered by the duct systems for mice treated with the two levels of oestrone were significant at the one per cent level; similar differences in the number of duct junctions were significant only at the ten per cent level.

The number of duct junctions per unit area in the mammae of ovariectomised mice appeared to be least in the group treated with 0.006 ug. of oestrone daily and greatest in the group not treated with oestrone. These overall differences were not significant*. The significant interaction between oestrone levels and replications appeared to be due solely to a discrepancy in the first replication (Table XXII) where the mean number of duct junctions per unit area was significantly lower for the oestrone-untreated group than for either of the oestrone-treated groups. In the other five blocks the means for the mice not injected with oestrone were consistently the greatest, while in all replicates the means for mice treated with the lower dose of oestrone were either larger than, or did not differ significantly from those for mice treated with 0.006 ug. daily.** The number of duct junctions per unit area for intact control mice was extremely variable and the

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* The appropriate "F" test of significance had the mean square due to interaction between blocks and oestrone levels, and not the residual mean square, as the denominator (see Table XXIII).

**Examination of differences between oestrone levels within each block was carried out by "t" test using the residual mean square as the estimate of variance.

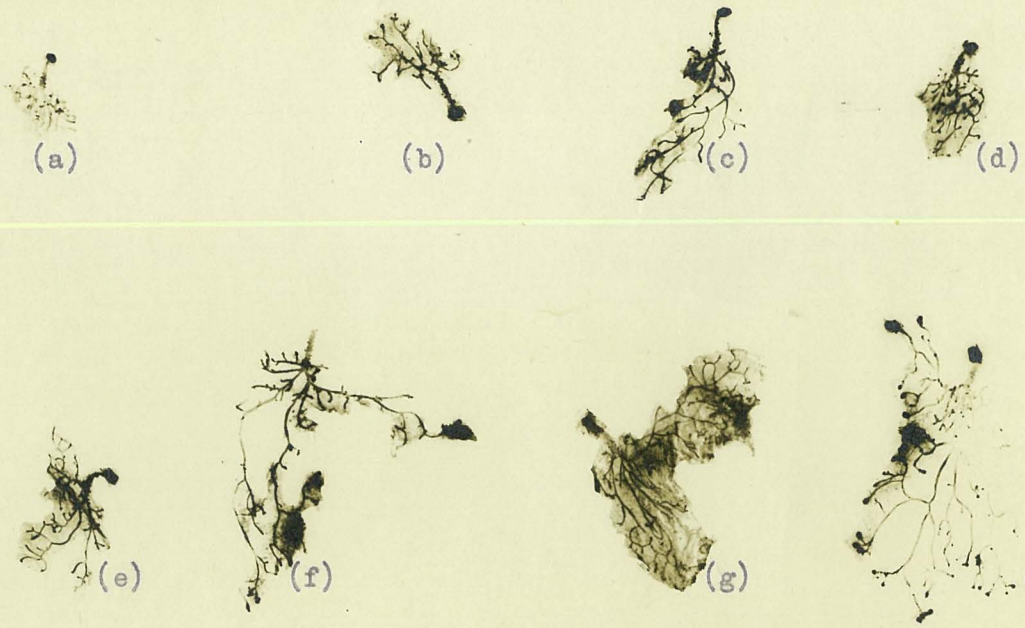

FIGURE 6. SECOND THORACIC MAMMARY GLANDS FROM OVARIECTOMISED ALBINO MICE TREATED WITH OESTRONE AND CORTISOL ACETATE (Magnification: 4X linear)

(a) 0.003 ug. oestrone and 50.0 ug. cortisol acetate daily
(b) " " " " 25.0 " " " "
(c) " " " " 12.5 " " " "
(d) " " "
(e) 0.006 " " " 50.0 " " " "
(f) " " " " 25.0 " " " "
(g) " " " " 12.5 " " " "
(h) " " "

FIGURE 7. THE THIRD THORACIC MAMMARY GLANDS OF A MOUSE TREATED WITH OESTRONE (0.003 ug. daily) (Magnification: 4 X linear)

mean value did not differ significantly from those for ovariectomised mice.

Whereas the two higher levels of cortisol acetate were without noticeable effect upon the mammae, treatment with 12.5 ug. of this steroid daily stimulated a slight but detectable degree of mammary development (Figure 6). This stimulation was apparent from the differences, in mean number of branches and mean area of the duct systems of the mammae, between cortisol-treated and cortisol-untreated ovariectomised mice, and between mice treated with 12.5 ug. daily and those injected with the two higher doses of cortisol (Table XIV).

The individual treatment means, when computed on the untransformed data, differed in a manner that suggested the effects of oestrone and cortisol were not simply additive. In the ovariectomised mice not treated with oestrone, all levels of cortisol appeared to have stimulated mammary development; in mice treated with the higher dose of oestrone (0.006 ug./day), the highest level of cortisol acetate (50 ug./day) seemed to have inhibited oestrone induced development (Table XIV). These discrepancies were reduced, however, in the case of the means estimated from the transformed data and on the logarithmic scale the variance due to interaction between the two steroids was negligible.

The estimates of the number of duct junctions per unit area for the mammae of ovariectomised mice did not appear to be affected consistently by cortisol treatment, although the overall mean for the mice treated with highest level of this steroid was slightly greater than those for the other groups of spayed mice.

Adrenal and Thymus Gland Weights

The weights of adrenal and thymus glands, obtained at autopsy, provided concomitant information on the activity of the cortisol acetate preparation at the levels administered. The direct thymolytic action of cortisol was more marked than its effect upon adrenal weight, which presumably was mediated by the anterior pituitary.

Oestrone administration was without apparent effect upon adrenal weight, but the complete removal of ovarian influence led to a significant degree of thymokesis.

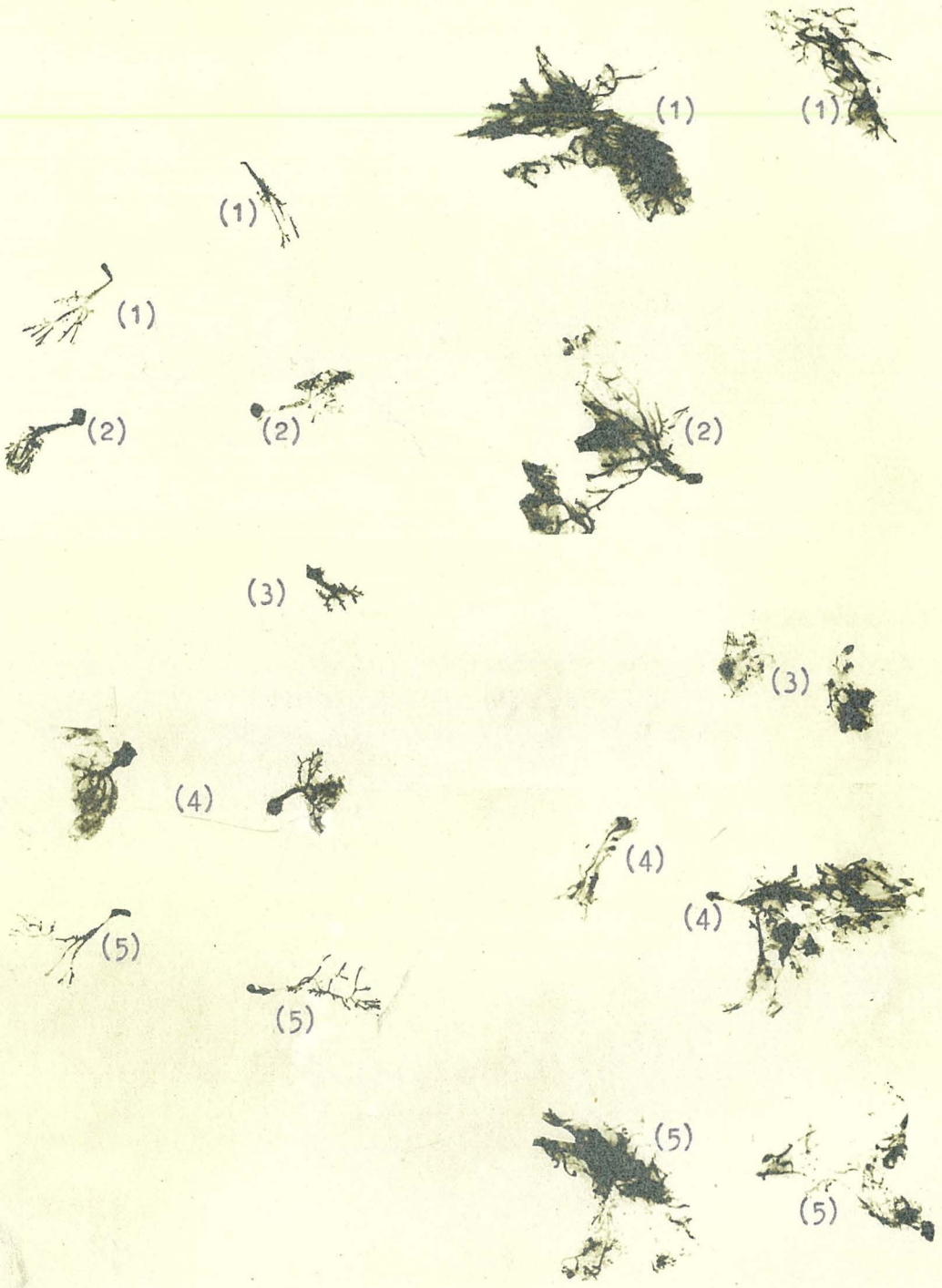

FIGURE 8. THE MAMMARY GLANDS OF TWO OVARIECTOMISED MICE TREATED WITH CORTISOL ACETATE (12.5 ug./day). (Magnification: 4 X linear)

The numerals in parentheses indicate the positions of the glands in the intact mouse. In the mouse on the left of the figure one of the third thoracic glands is absent; in the other mouse one of the second thoracic glands was destroyed during the process of dissecting the mammary glands free of the skin.

This was apparent from the significant difference between intact control and spayed control mice, and from the significant interaction between oestrone and cortisol levels.

Uterus Weight and Vaginal Response

The uterine weight at slaughter, the time of vaginal introitus and the vaginal changes following introitus were recorded as a check on the level of oestrogenic stimulation.

The response of the uterus to oestrone followed a pattern similar to that of the mammae. Cortisol acetate was without significant effect upon uterine weight, although at each level of oestrone administration the uterine weights of cortisol-treated mice were collectively slightly greater than those of mice not treated with this steroid.

In the case of vaginal introitus the response, both as regards the proportion of animals responding and the period of treatment elapsing prior to introitus, to the two doses of oestrone differed significantly. The response in mice treated with the higher level of oestrone could not be distinguished, however, from that obtaining in intact mice.

The vaginal smears of the oestrone-treated ovariectomised mice did not show evidence of oestrus chnages in the vaginal wall. Leucocytes were present in significant numbers in every smear from all of the ovariectomised mice showing introitus, with the exception of one mouse which was subsequently excluded from consideration by reason of its abnormally large uterus. Normal cyclic vaginal cornification was detected in five of the six intact mice; in the sixth mouse leucocyte-free smears occurred twice within five days of introitus, but were not subsequently detected.

Cortisol acetate administration did not affect the numbers of animals exhibiting vaginal introitus. The effect of this steroid upon the time elapsing prior to vaginal opening was not examined statistically.

Discussion

The Effects of Ovariectomy and Oestrone Administration

The effect of ovariectomy and of the subcutaneous injection of oestrone on the mammae and reproductive tract of albino mice were qualitatively similar to those reported with other strains of mice (Flux, 1953, 1954; Mühlbock, 1948, 1953).

The experimental procedure and criteria of response employed in the present study were based on those used earlier by Flux (1953) with CHI mice; therefore, approximate comparison of the oestrone sensitivities of albino and CHI mice was feasible. The lowest level of oestrone administration reported by Flux (1953, 1954) was 0.01 ug. daily. The increase in area of the mammae of ovariectomised CHI mice treated with this level of oestrone, in the course of several experiments, was approximately half as great as that obtaining in untreated intact mice. Albino mice treated with 0.006 ug. oestrone daily were found to have a mean mammary area about one third as great as that of intact mice (Table XIV). It seemed, therefore, that mice of the albino strain were slightly more sensitive to oestrone , in respect of growth in area of the mammae, than CHI mice. A similar conclusion could be drawn with regard to the increase in number of duct branches in the mammary glands, but in the case of the uterine weight response it appeared that the CHI strain might be the more sensitive. In view of the very considerable variation in the response of individual mice accorded similar treatment, which was observed in the present experiment and by Flux (1953), little reliancecould be placed on these slight strain differences. Indeed the quantitative response of the mammae of these two strains of mice, when contrasted with the ten fold difference in sensitivity found between mice of the 020 and "Riet" strains by Mühlbock (1948), was remarkably similar.

Although direct comparisons of the oestrone-sensitivity of albino mice with

those of the strains of mice investigated by Muhlbock (1948, 1953) were not feasible, because of differences in the criteria used to evaluate response and in the period of oestrone treatment, the relative sensitivities of the different organs could be compared. In albino mice it appeared (Table XIV) that vaginal introitus was most readily, and vaginal cornification least readily stimulated by the subcutaneous injection of oestrone. The oestrone-sensitivities of the mammae and uteri were intermediate. The results obtained (Muhlbock, 1948, 1953) with immature ovariectomised mice of the C57 Bl, CBA, A, DBA, and 020 strains suggested that the amount of parenterally administered oestrone required to produce oestrous symptoms was greater than that needed for minimal stimulation of the mammae. This discrepancy was most marked in DBA mice, in which the vagina appeared relatively insensitive to oestrone. In mice of a further strain—the "Riet" strain—in which the mammae were comparatively oestrone-insensitive, the discrepancy between the sensitivity of the vagina and mammary gland was not apparent.

The Effect of Cortisol Acetate on the Mammae

In the present study the lowest level of cortisol acetate injected caused a slight but significant degree of mammary development, whereas the higher levels of this steroid were without significant effect on the mammae. When the response of the mammae was examined on a logarithmic scale, the effects of cortisol appeared additive with those of oestrone, in so far as the variance due to interaction between the different levels of the two steroids was negligible.

Flux (1953, 1954) reported inhibition of normal and oestrone-induced mammary growth in CHI mice as a result of injection of 11-oxygenated steroids. The lowest level of cortisol acetate used by this worker was 100 ug. daily, and at this dosage noticeable inhibition of mammary gland growth was observed in ovariectomised mice treated with 0.01 ug. oestrone daily. The results obtained with lower levels of this steroid in albino mice were compatible with those described by Flux, particularly since at the daily level of 50 ug. cortisol acetate appear-

ed to have reduced the reponse of the mammae of ovariectomised albino mice to the 0,006 ug. level of oestrone. This effect was less noticeable when the response was examined on the logarithmic scale and was not of sufficient magnitude to produce a significant interaction (Table XIV) between the levels of the two steroids.

Cortisol and cortisone have been claimed to stimulate mammary development in the rat (Johnson and Meites, 1955; Selye, 1954a,b). The basis for these claims has been examined critically in an earlier part of this thesis (Cf. ante, pp. 32, 33) both with regard to the criteria used to evaluate the response of the mammae and in respect of the use of only one level of cortisol. The results obtained with mice substantiate the earlier criticism. The contention, that any generalisation regarding the effect of cortisol or cortisone on the mammary gland must be supported by results of treatment with more than one level of steroid, was emphasized by the contrasting effects of cortisol at different dosages in ovariectomised mice. Flux (1953, 1954) drew attention to the advisibility of considering growth in area and increase in duct branching separately, when examining the effect of any treatment on mammary gland growth. The results obtained in the present experiment support the contention that inhibition and stimulation of the mammae cannot be distinguished, when the consideration of the density of duct branching (number of duct junctions per unit area) is the sole criterion of the response of the mammae.

The Effect of Cortisol Acetate on Body and Organ Weights

The effects of cortisol on body growth and on the weights of adrenal and thymus glands observed in the present investigation were similar to the effects of 11-oxygenated adrenal steroid administration described by other workers (see Noble, 1950, 1955). The effect of cortisol acetate upon the thymus glands of CHI mice observed by Flux (1954) was greater than was found with albino mice in the present experiment. This was not surprising in view of the higher levels of the steroid administered to the CHI mice. In so far as inhibition of ACTH release and body growth were concerned, it seemed that albino mice were more

sensitive to cortisol acetate (Table XIV) than were CHI mice (Flux, 1954).

Cortisol acetate was without significant effect upon uterine weight in the present study, though the weights of the uterus were higher in cortisol-treated mice as a group than in cortisol-untreated ovariectomised mice. This small non-significant difference was of interest in view of the observation that cortisone acetate at somewhat higher levels increased the uterine weight gains of ovariectomised CHI mice treated with 0.02 ug. oestrone daily (Flux, 1954). A similar effect could not be demonstrated with this steroid, or with cortisol, corticosterone or dehydrocorticosterone in mice treated with 0.01 ug. oestrone daily. Other workers have observed partial suppression of oestrogen-induced uterine weight increases in ovariectomised mice and rats treated with cortisol acetate (Szego and Roberts, 1953; Talalay, Dobson, Ebersole and Huggins, 1952).

It seemed likely that the observation of any effect of 11-oxygenated corticosteroids on oestrogen-induced uterine weight increase depended upon the absolute and relative levels of the two types of steroid, and that the nature of the change occurring in the uterine tissue might also determine the direction of any weight change. In the present investigation, and in that reported by Flux (1954), only the weights of the cleaned, split and blotted uteri was recorded so that any changes observed might have reflected only a greater tissue water content. Szego and Roberts (1953) , however, demonstrated that the suppression of oestrogen-induced uterine weight increase, which they observed as a result of cortisol acetate administration, was concerned with true tissue growth and did not merely reflect changes in the water content of the tissues.

Summary

A difference in the degree of development of the mammary glands--both the area of, and number of junctions in the duct systems--of ovariectomised albino mice treated with 0.003 ug. and 0.006 ug. oestrone daily, could be distinguished after twenty one days of treatment. Differences between oestrone-treated ovariectomised mice, and both ovariectomised and intact control mice were also significant. Similarly, four levels of ovarian influence could be detected in the weight response of the uterus. In the case of vaginal introitus the effects of the lower level of oestrone could be distinguished from those of the higher dose. The response to the latter, however, was indistinguishable from that obtaining in the presence of the ovary. Vaginal smears characteristic of oestrus--absence of leucocytes--were not detected in oestrone-treated ovariectomised mice. The cyclic occurrence of vaginal cornification was observed in five of the six intact untreated mice. Neither ovariectomy nor oestrone administration affected the rate of body growth or the adrenal gland weight at slaughter. Ovariectomy promoted a significant degree of thymokesis.

When injected at a level of 12.5 ug. daily for 21 days, cortisol acetate stimulated mammary development in ovariectomised and ovariectomised, oestrone-treated albino mice, both growth in area and increase in the number of duct junctions being affected. At higher dosage rates (25 ug. and 50 ug. daily) this steroid was without detectable influence on the mammae. All levels of cortisol acetate caused a degree of thymolysis and inhibited body weight increase. These effects were most marked in mice injected with 50 ug. daily, in which mice adrenal weight was also noticeably reduced.

The effect of the two steroids upon the mammae appeared to be independent, at least when analysed on the transformed (logarithmic) scale.

The estimate of the density of duct branching--number of duct junctions per

unit area--was not significantly affected by either steroid. Differences in this estimate of mammary gland structure were in general inversely related to differences in the area or number of duct junctions.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The results obtained in the several experiments reported in this thesis have been summarised at the end of each chapter or section of a chapter. In the following pages the more important observations have been recapitulated and an attempt made to draw a number of general conclusions. The latter, because of the preliminary nature of the experiments described, tend to take the form of suggestions of possible avenues of future investigation.

A total of more than 300 albino mice, together with a small number of mice of three other strains (CBA, AW and NZC), were used in studies of the factors affecting survival after bilateral adrenalectomy. The majority of adrenalectomised mice died within a week of operation; a small proportion survived indefinitely. The most important determinants of the lifespan of adrenalectomised mice of the albino strain were: the completeness of adrenal ablation; the rate of hypertrophy and the occurrence of accessory cortical tissue; the presence or absence of treatment with cortisol acetate; age at the time of operation, and "individuality". Sex, gonadectomy and the provision of additional sodium chloride had little or no influence upon the survival of mice of this strain. A small proportion of adrenalectomised mice of the CBA and AW strains were enabled to survive so long as one per cent of sodium chloride was added to the drinking water.

Cortisol acetate promoted survival in adrenalectomised male albino mice at levels ranging from 12.5 ug. to 75 ug. daily. A greater proportion of mice survived at the higher dosage levels and the body growth rate of these animals was more nearly normal than that of mice enabled to survive by treatment with lower doses of the steroid. Treatment with cortisol acetate revealed the presence of accessory cortices, not detected in untreated mice, that were reflected in the survival of a proportion of adrenalectomised mice long after treatment with the

adrenal steroid ceased. The occurrence of indefinite survival was greater in mice treated with the higher levels of cortisol.

The effect of cortisol acetate upon the mammae of ovariectomised and ovariectomised, oestrone-treated albino mice was investigated using the levels of steroid found effective in life-maintenance in adrenalectomised male mice of the same age. The lowest level (12.5 ug. daily) of cortisol acetate stimulated slight but significant growth of the mammary gland. The two higher levels (25 ug. and 50 ug. daily) were without significant effect on the mammae.

Of the strains of mice studied, the most suited to investigation of the effect of total adrenal insufficiency on the mammae appeared to be the CBA. In this strain sodium chloride was partially effective in promoting survival, and accessory cortical tissue did not attain functional significance, if in fact it was present in mice of this strain. Mice of the AW strain, although a proportion were favourably influenced by the addition of sodium chloride to the drinking water, were not suited to any study involving total adrenal insufficiency because of the frequent occurrence of accessory cortices of functional importance. Further studies with CBA mice appeared to be warranted; the effect of special diets, in which the levels of potassium and sodium salts were rigidly controlled, upon survival would be of particular in relation to the use of these mice in studies requiring the maintenance of totally adrenal insufficient animals.

Immature albino mice appeared to be suited to studies of mammary gland growth where it was desired to exclude any effect mediated by changes in the adrenal cortical secretion, provided that the level of cortisol replacement therapy was chosen so as to minimise the promotion of indefinite survival. Doses of the order 12.5 ug to 25 ug. daily appeared to meet this proviso best. Further studies of the effect of these low levels of cortisol acetate on the mammae are, however, required in order that a level of the steroid can be selected which does not of itself affect mammary development. These studies would need to be carried out

ovariectomised, adrenalectomised and ovariectomised-adrenalectomised mice to provide a complete answer to the problem.

There is a place in studies involving adrenalectomy and replacement therapy for a convenient physiological test of the presence of accessory or residual cortical tissue. Histological methods are limited in their applicability by the need to examine serial sections of a very large part of the body of each mouse. When restricted to the region surrounding the principal adrenal glands histological examination is by no means an infallible test of the presence of adrenal rests. Those existing indices of adrenal cortical function which involve subjecting the adrenalectomised animal to some form of stress will not serve to detect accessory cortices that, although adequate for the protection of the animal in a "neutral" environment, do not afford protection against that form of stress. Although the contribution of such undetected accessories might not be very large, it could result in erroneous conclusions were the study concerned with the mammary gland and were albino mice of the strain used in the present investigation the experimental animals. A test based upon the ability of ACTH to produce eosinopenia might suffice to detect accessory cortical tissues in adrenalectomised animals. In its present form—that suggested by Speirs and Meyer (1949)—this test appears to the author to be too involved and too tedious for rapid routine application.

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A P P E N D I X

A. Individual Survival Periods (days)

	21 - 25 DAYS		40 - 45 DAYS		84 - 90 DAYS	
	WATER	SALINE	WATER	SALINE	WATER	SALINE
FEMALE	7	7	3	7	3	5
	3	4	5	9	2	9
	3	4	5	5	4	6
	3	5	3	2	4	5
	6	3	5	6	5	8
	4	3	5	7	12	15
	4	4	5	6	12	8
	3	7	4	6		
	7	3	3	6		
	8	5	8	9		
OVARIECTOMISED FEMALE	4	7	2	5	8	4
	2	5	5	3	3	5
	2	2	4	3	3	5
	2	4	3	3	2	3
	4	2	3	5	6	15
	3	3	8	5	12	7
	3	2	5	7	11	8
	2	2	10	5		
	3	2	8	5		
	3	2	3	7		
MALE	5	5	3	5	4	4
	2	2	3	7	4	5
	3	3	5	4	7	5
	3	3	5	4	6	5
	3	2	5	4	8	3
	2	2	5	5	9	16
	3	4	5	6	10	12
	3	4	4	4		
	3	3	5	3		
	3	2	5	10		
CASTRATE MALE	7	4				
	3	4				
	4	2				
	4	3				
	3	2				
	3	2				
	2	4				
	2	4				
	6	4				
	3	2				

TABLE "X"

TABLE XV

THE SURVIVAL TIMES OF ALBINO MICE ADRENALECTOMISED AT THREE AGES: THE EFFECT OF SEX, GONADECTOMY, AND ADMINISTRATION OF SODIUM CHLORIDE

B. Analyses of Variance of Survival Times After Transformation[1]

SOURCE OF VARIATION	21 - 25 DAYS OF AGE AT OPERATION			40 - 45 DAYS OF AGE AT OPERATION			84 - 90 DAYS OF AGE AT OPERATION		
	DEGREES OF FREEDOM	MEAN SQUARE	"F"	DEGREES OF FREEDOM	MEAN SQUARE	"F"	DEGREES OF FREEDOM	MEAN SQUARE	"F"
BLOCKS	9	65991	1.36^{-}	9	435841	2.78*	6	213707	6.45**
DRINKING SOLUTIONS	1	11737	<1	1	54843	3.15^{+}	1	46141	1.40^{-}
SEX AND OPERATIONS	3	158450	11.01**	2	13603	<1	2	8939	<1
BETWEEN SEXES	1	79569	5.53*						
"INTACT" v. SPAYED FEMALE	1	377719	26.24**						
"INTACT" v. CASTRATE MALE	1	18063	1.25^{-}						
BLOCKS X DRINKING SOLUTIONS	9	48567	3.37*	9	29977	1.72^{-}	6	24305	<1
BLOCKS X SEX AND OPERATIONS	27	13788	<1	18	21298	1.22^{-}	12	25278	<1
DRINKING SOLNS. X SEX AND OPERATIONS	3	3698	<1	2	18848	1.08^{-}	2	40854	1.24^{-}
RESIDUAL	27	14394		18	17416		12	32940	
TOTAL	79			59			41		

** Highly Significant ($p < 0.01$); * Significant ($0.01 < p < 0.05$); $^{+}$ Not Significant ($0.10 \geq p \geq 0.05$); $^{-}$ Not Significant ($p > 0.10$).

[1] The data were transformed according to the relation: X = 1000 log days to stabilise the subclass variances.

TABLE XVII

DISTRIBUTION OF DEATHS DUE TO ADRENAL INSUFFICIENCY IN ADRENALECTOMISED IMMATURE MALE AND FEMALE ALBINO MICE

			Survival period (days)						
			2	3	4	5	6	7	Total
Number of Mice	Female:	Saline	1	3	3	2	2	-	11
		Water	2	3	4	3	4	-	16
	Male:	Saline	3	6	4	2	1	1	17
		Water	1	7	2	2	1	1	14

TABLE XVIII

DISTRIBUTION OF DEATHS DUE TO ADRENAL INSUFFICIENCY IN ADRENALECTOMISED IMMATURE MALE NZC MICE

		Survival Period (days)						
		2	3	4	5	6	...12	Total
Number of Mice	Saline	-	3	3	2	-	1	9
	Water	3	3	4	3	2	-	15

TABLE XVI

TABLE XVI

ANALYSIS OF VARIANCE* OF THE LIFESPAN OF IMMATURE ADRENALECTOMISED ALBINO MICE:

THE EFFECT OF SEX AND SODIUM CHLORIDE ADMINISTRATION

SOURCE OF VARIATION	D.F.	MEAN SQUARE	"F"
Male v. Female	1	2.1411	1.12 N.S.
Water v. Saline	1	0.3393	1
Interaction	1	0.00035	1
Residual	54	1.9084	

* Analysis carried out on the untransformed data by the method described by Snedecor (1946, pp. 285 et seq.) as appropriate where the interaction between treatments was negligible.

N.S. : Not significant ($p > 0.05$).

TABLE XIX (Contd)

Oestrous Changes in Females

+ Complete absence of oestrous-type vaginal smears
*** Several cycles with prolonged dioestrous phases
** One or two cycles with interval between successive oestrous smears either normal(?) or shortened
* Normal pattern of cyclical occurrence of vaginal cornification

General Symbols

+ Indefinite survivor
- Not available, usually because the animal was dead.
Shk: death occurred with a few hours of the operation, classified as due ot post-operative shock.

TABLE XIX

DISTRIBUTION OF DEATHS AND INDEFINITE SURVIVAL; BODY WEIGHTS, AND THE OCCURRENCE OF VAGINAL CORNIFICATION IN ADRENALECTOMISED MATURE MICE OF THE CBA AND AW STRAINS

DRINKING SOLUTION: WATER					DRINKING SOLUTION: WATER + 1% NaCl				
MOUSE	LIFE-SPAN (days)	BODY WEIGHT (gm) at operation	at death	at 21 days	MOUSE	LIFE-SPAN (days)	BODY WEIGHT (gm) at operation	at death	at 21 days
				CBA FEMALE MICE					
1*+*	8	16.9	14.4	-	3*+*	9	16.3	15.2	-
2**	8	18.2	15.5	-	10***	11	17.8	14.9	-
11**	7	18.8	15.3	-	20***	26	20.2	17.5	20.3
50**	8	20.0	17.7	-	21***	16	18.6	15.8	-
102*+*	6	18,5	15.5	-					
				AW FEMALE MICE					
3**	11	19.1	15.7	-	4*+*	5	19.8	15.9	-
5*+*	20	17.7	15.2	-	15**	7	18.6	14.5	-
6**	14	18.6	14.5	-	102*+*	7	17.5	13.3	-
13**	11	18.8	15.2	-	103*	+	20.2	[illegible]	20.0
16*+*	12	22.0	16.8	-	109**	5	16.3	13.5	-
101*+*	9	16.9	14.2	-	110**	5	17.5	14.2	-
104*	+	17.3	-	19.6	118*+*	5	15.6	13.0	-
105*+*	8	15.7	12.3	-					
108*+*	8	16.7	13.0	-					
				CBA MALE MICE					
1	6	20.4	18.0	-	2	11	20.0	16.5	-
3	6	21.3	19.0	-	12	14	23.4	18.5	-
14	10	26,3	21.8	-	16	11	26.8	21.5	-
22	15	23.9	19.5	-	20	26.2	26.2	19.5	27.3
23	14	25.3	20.0	-	21	13	25.2	20.5	-
				AW MALE MICE					
6	+	14.7	-	15.2	1	+	17.1	-	20.5
7	3	17.3	14.7	-	2	25	16.1	14.0	17.4
8	5	18.9	15.5	-	10	+	14.5	-	21.5
9	+	14.5	-	21.0	23	5	20.8	17.5	-
20	Shk	21.2	-	-	25	Shk	23.0	-	-
21	10	22.5	18.8	-	102	+	19.6	-	23.6
24	Shk	21.7	-	-	103	26	17.5	16.0	19.7
105	3	15.9	12.6	-	107	14	18.4	14.3	-
159	+	17.5	-	20.5	109	30	15.8	17.5	19.4

[illegible] and symbols see opposite page.

TABLE XX

THE EFFECT OF SODIUM CHLORIDE AND CORTISOL ACETATE ADMINISTRATION UPON THE SURVIVAL AND BODY WEIGHT OF BILATERALLY ADRENALECTOMISED MALE ALBINO MICE

LEVEL OF CORTISOL ACETATE (ug.)	LEVEL OF ADDED SODIUM CHLORIDE IN DRINKING FLUID (%)											
	0				1				2			
	REPLICATION				REPLICATION				REPLICATION			
	1	2	3	4	1	2	3	4	1	2	3	4
	LIFESPAN: DAYS FROM OPERATION											
12.5	27	+	7	25	25	24	12	11	+	25	26	26
25.0	12	9	11	27	11	26	24	24	+	25	25	23
50.0	26	+	25	+	+	+	+	24	28	17	+	25
75.0	27	+	31	+	28	+	30	+	26	+	26	25
CONTROL**	+	+	+	+	+	+	+	+	+	+	+	+
	BODY WEIGHT AT OPERATION (gm.)											
12.5	12.3	10.2	10.5	12.1	11.8	9.2	10.9	11.0	11.5	9.5	12.5	10.5
25.0	11.4	10.8	10.2	9.3	11.6	19.4	11.4	13.6	11.3	9.8	11.0	11.1
50.0	13.3	10.2	10.7	10.0	11.9	10.7	10.0	11.8	10.3	13.2	10.0	14.5
75.0	10.7	9.7	14.2	9.5	11.3	11.0	11.0	11.2	10.0	11.2	11.7	11.5
CONTROL**	12.7	13.2	13.0	14.2	10.5	9.7	14.0	12.6	11.8	10.2	11.5	9.2
	BODY WEIGHT: 21 DAYS AFTER OPERATION (gm.)											
12.5	17.8	17.9	8.9*	14.7	16.0	12.2	10.5*	10.4*	14.0	11.8	16.6	12.9
25.0	10.3*	10.0*	9.4*	12.5	10.3*	15.8	15.8	16.1	14.7	15.1	15.4	14.9
50.0	18.8	18.2	15.9	14.6	20.0	21.8	15.2	15.6	14.4	13.0*	15.1	18.7
75.0	16.2	15.6	20.8	14.6	16.1	15.7	14.1	15.2	12.6	18.3	16.2	15.3
CONTROL**	22.2	17.3	13.3	18.9	16.9	19.3	21.6	13.8	19.2	19.5	17.1	12.3

* Body weight immediately prior to death (within preceding 24 hours); mice not surviving throughout the treatment period.

**Control mice subjected to unilateral adrenalectomy; not treated with cortisol acetate.

+ Mice not dying within twenty one days of the cessation of treatment; classified as indefinite survivors.

TABLE XXI

TABLE XXI

ANALYSES OF VARIANCE OF THE LIFESPAN, BODY WEIGHT GAIN, AND REGRESSION OF BODY WEIGHT GAIN ON THE LOG-DOSE OF CORTISOL FOR ADRENALECTOMISED MALE ALBINO MICE, MAINTAINED BY THE INJECTION OF CORTISOL ACETATE.

	ALL MICE DYING			MICE DYING AFTER TREATMENT CEASED				
	LIFESPAN[1.] (log.days)				LIFESPAN[2.] (log.days)		BODY WEIGHT GAIN (gm)	
Source of Variation	d.f.	Mean Square	"F"	d.f.	Mean Square	"F"	Mean Square	"F"
Total	33			25				
Between Treatments	11	0.03440	1.30^{ns}	11	0.03859	2.79*	1.580	1.18^{ns}
Cortisol levels				3	0.07006	5.06*		
"Salt" levels				2	0.03937	2.84^{ns}		
Interaction				6	0.02259	1.63^{ns}		
Within Treatments	22	0.02639		14	0.01385		1.336	

REGRESSION OF BODY WEIGHT GAIN ON LOG-DOSE CORTISOL MICE DYING AFTER TREATMENT CEASED			
Source of Variation	d.f.	Mean Square	"F"
Total	25		
Between Cortisol Levels	3		
Due to Regression	1	4.037	2.96^{ns}
Dev. from Regression	2	1.016	1
Within Cortisol Levels	22	1.364	

1. Lifespan measured from the time of adrenalectomy
2. Lifespan after the cessation of injections of cortisol acetate

* Significant, $p < 0.05$

ns Not significant, $p > 0.05$.

TABLE XXII

THE EFFECT OF CORTISOL ACETATE AND OESTRONE UPON THE BODY WEIGHT, THE MAMMAE, AND OTHER ORGANS OF OVARIECTOMISED ALBINO MICE

BLOCK	DOSE OF CORTISOL ACETATE (ug./day)												INTACT CONTROL MICE
	NIL			12.5			25.0			50.0			
	DOSE OF OESTRONE (ug./day)												
	NIL	.003	.006	NIL	.003	.006	NIL	.003	.006	NIL	.003	.006	
	MAMMAE: TOTAL AREA ($mm.^2$)												
1	33.8	81.0	347.8	84.4	92.1	230.4	127.2	77.4	173.4	81.5	99.1	79.7	514.2
2	40.2	79.7	86.7	44.9	44.4	120.2	45.4	98.0	131.6	57.5	110.4	116.9	456.1
3	32.8	104.7	127.2	30.4	126.9	212.6	50.8	71.7	175.2	29.4	49.0	93.1	258.8
4	75.1	111.2	184.2	44.1	138.5	249.7	43.3	85.4	163.6	40.5	74.3	295.2	279.9
5	55.5	80.0	122.8	161.0	106.0	407.4	41.8	105.3	97.3	72.5	55.2	59.6	500.0
6	61.4	59.3	150.7	87.2	290.8* (117.7)	289.5	70.4	134.9	238.9	64.0	99.6	110.9	665.9
	MAMMAE: NUMBER OF DUCT JUNCTIONS												
1	57	197	383	116	170	231	138	136	253	114	241	149	615
2	84	123	161	82	96	226	98	99	232	86	171	204	639
3	68	194	245	80	184	366	107	110	124	75	116	160	686
4	120	140	210	89	218	276	50	115	231	90	111	351	610
5	56	86	160	199	173	328	89	152	149	78	105	88	534
6	68	86	230	182	257* (169)	230	128	132	234	97	127	194	1457
	MAMMAE: DUCT JUNCTIONS PER UNIT AREA (./$mm.^2$)												
1	4.52	4.55	2.93	2.57	5.48	3.48	2.74	5.60	2.76	2.39	3.78	4.01	2.90
2	5.92	2.92	3.09	4.18	4.53	5.89	5.88	3.68	4.83	4.72	2.54	3.53	3.20
3	5.71	4.91	3.70	6.61	2.59	2.85	5.12	3.38	2.43	7.43	4.33	4.88	5.09
4	3.32	2.52	2.10	5.86	3.53	3.45	4.95	2.73	2.69	5.59	4.07	3.05	4.44
5	3.97	1.92	3.28	2.47	2.58	2.37	4.78	2.92	3.07	2.69	4.20	3.25	2.32
6	3.33	4.70	2.55	3.90	2.13* (3.27)	2.22	3.03	3.26	2.37	3.80	3.79	4.50	7.60
	INITIAL BODY WEIGHTS (gm)												
1	9.2	14.6	11.1	12.3	12.5	13.3	12.6	10.2	10.5	14.5	12.5	14.6	11.4
2	9.6	11.5	12.4	10.2	9.6	8.8	10.8	10.8	10.2	11.0	16.2	10.7	11.6
3	7.1	10.1	9.2	11.7	10.4	12.7	8.5	11.0	7.5	7.5	8.0	11.2	7.0
4	8.6	8.0	10.7	11.7	9.3	8.8	9.7	10.3	10.0	8.7	12.0	9.6	11.7
5	8.9	8.1	9.3	9.8	8.5	9.5	8.8	12.1	9.5	9.3	7.7	9.2	10.0
6	7.2	8.5	10.2	8.4	11.4* (8.8)	8.6	8.8	8.1	10.0	10.6	9.0	11.5	9.2
	SLAUGHTER BODY WEIGHTS (gm)												
1	17.3	20.2	17.5	19.7	15.6	18.1	19.0	17.6	15.7	17.2	17.4	17.5	19.5
2	18.2	20.0	20.0	17.7	15.7	15.5	17.8	17.8	16.2	15.9	19.3	18.3	18.2
3	16.9	16.5	16.2	16.1	18.5	17.4	15.0	16.5	14.3	13.9	15.5	14.9	15.5
4	18.2	16.8	18.1	17.4	15.4	15.7	15.8	16.4	17.8	15.4	16.1	14.3	17.8
5	16.5	16.7	16.2	16.2	15.8	15.9	14.4	17.2	15.4	14.7	14.2	14.6	19.0
6	13.5	16.0	19.6	13.7	16.5* (14.6)	14.5	14.5	14.5	17.3	14.3	13.6	13.4	16.0

(Continued on the following page.)

TABLE XXII (CONTINUED)

BLOCK	DOSE OF CORTISOL ACETATE (ug./day)												INTACT CONTROL MICE
	NIL			12.5			25.0			50.0			
	DOSE OF OESTRONE (ug./day)												
	NIL	.003	.006	NIL	.003	.006	NIL	.003	.006	NIL	.003	.006	
	UTERUS WEIGHT (mg.)												
1	5.5	9.0	8.0	5.0	5.5	7.0	6.0	6.0	10.0	5.5	8.0	9.0	83.0
2	5.0	8.0	9.0	5.0	6.5	8.0	5.5	7.0	8.0	6.0	7.0	8.0	19.5
3	5.5	5.5	7.0	6.5	7.5	6.5	5.5	5.5	7.5	5.0	4.0	8.0	31.5
4	6.5	5.0	7.5	5.0	8.5	10.5	5.5	6.0	6.0	4.5	5.0	9.5	19.5
5	3.5	5.0	5.5	9.0	7.5	8.0	6.5	6.5	7.0	4.0	6.5	7.5	57.5
6	4.0	6.0	9.0	6.5	12.5* (8.0)	10.5	7.0	7.0	10.0	5.5	6.5	10.0	35.0
	ADRENAL WEIGHT (mg.)												
1	5.0	5.5	5.5	5.5	7.0	5.5	6.5	6.0	6.0	5.5	6.0	6.0	5.0
2	6.0	7.0	6.5	7.0	6.0	6.0	5.5	5.0	5.5	6.0	7.0	6.0	5.5
3	6.0	7.5	6.0	6.0	4.5	5.5	6.0	5.0	4.0	4.5	6.0	5.0	7.0
4	6.5	6.0	6.0	7.0	6.0	5.5	4.5	6.0	5.5	6.5	4.5	5.0	5.5
5	6.5	6.5	6.5	5.0	5.5	6.0	5.0	5.5	5.0	6.0	5.0	5.0	6.0
6	6.5	5.0	7.5	5.5	5.5* (5.5)	5.5	4.5	5.0	5.0	5.5	4.5	4.5	6.0
	THYMUS WEIGHT (mg.)												
1	66.0	54.0	67.0	67.5	41.5	53.5	57.5	62.0	51.5	43.5	40.5	33.5	59.0
2	75.0	60.0	71.5	41.5	65.5	52.0	53.5	53.5	42.0	56.5	31.5	59.5	61.5
3	75.0	69.0	45.5	45.5	72.5	42.5	41.5	44.0	47.5	48.0	31.0	42.0	65.5
4	68.5	62.5	64.5	31.0	40.5	43.5	42.0	59.5	60.5	41.0	30.0	36.5	62.5
5	71.5	53.5	41.5	46.0	61.0	49.5	42.0	37.0	33.5	43.0	26.5	29.5	48.0
6	71.0	58.5	51.5	33.5	42.5* (47.5)	27.5	34.0	31.5	39.5	14.0	21.5	13.0	45.5
	TIME OF VAGINAL OPENING (days)												
1	-	18	17	-	16	15	-	-	18	-	14	13	15
2	-	15	7	-	-	20	-	-	12	-	4	14	10
3	-	20	14	-	-	7	-	10	16	-	15	12	18
4	-	-	16	-	17	9	-	16	12	-	8	10	12
5	-	-	12	-	21	13	-	20	10	-	15	10	8
6	-	-	14	-	12* (17)	15	-	14	13	-	17	13	13

* These data were excluded on the basis of the outlying uterus weight and were replaced by values (shown in parentheses) estimated by missing plot analyses.

TABLE XXIII

ANALYSES OF VARIANCE OF ESTIMATES OF EXTENT AND DENSITY OF THE MAMMARY GLAND DUCT SYSTEM OF OVARIECTOMISED MICE TREATED WITH OESTRONE AND CORTISOL ACETATE

SOURCE OF VARIATION	DEGREES OF FREEDOM	TOTAL GLAND AREA ($\log_{10}$ mm.^{2})		NUMBER OF DUCT JUNCTIONS[2.] ($\log_{10}$ total)		($\log_{10}$ total/mm.^{2})	
		MEAN SQUARE	F	MEAN SQUARE	F	MEAN SQUARE	F
Blocks	5	0.1019	3.84 **	0.0238	1.61 NS	0.03895	1.16 NS
Oestrone Levels	2	1.4861	55.95 **	0.7938	53.56 **	0.08364	2.49 NS
Treated v. Untreated	1	1.9258	72.51 **	1.1565	78.14 **		
Dose 0.003 v. 0.006 ug.	1	1.0464	39.40 **	0.4311	2.91 +		
Cortisol Levels	3	0.0595	2.24	0.0692	4.67 **	0.00798	<1
Treated v. Untreated	1	0.0548	2.06 NS	0.0345	2.33 NS		
Dose 12.5 v. 25.0 + 50.0 ug.	1	0.1146	4.32 *	0.1720	11.62 **		
Dose 25.0 v. 50.0 ug.	1	0.0092	<1	0.0012	<1		
Blocks x Oestrone Levels	10	0.0437	1.64 NS	0.0133	<1	0.03360	3.55 **
Blocks x Cortisol Levels	15	0.0478	1.80 NS	0.0289	1.95 +	0.01750	1.85 +
Oestrone Levels x Cortisol Levels	6	0.0091	<1	0.0148	1.00	0.00659	<1
Blocks x Oestrone x Cortisol Levels	29[1.]	0.0266		0.0148		0.00948	

TABLE XXIV

ANALYSES OF VARIANCE OF GLAND, ORGAN, AND BODY WEIGHTS OF OVARIECTOMISED MICE TREATED WITH OESTRONE AND CORTISOL ACETATE FROM 21 TO 42 DAYS OF AGE

SOURCE OF VARIATION	DEGREES OF FREEDOM	UTERUS WEIGHT (mg.)		THYMUS WEIGHT (mg.)		ADRENAL WEIGHT (mg.)		INITIAL BODY WEIGHT (g.)		GAIN IN BODY WEIGHT (g.)	
		MEAN SQUARE	F	MEAN SQUARE	F	MEAN SQUARE	F	MEAN SQUARE	F	MEAN SQUARE	F
Blocks	5	2.95	1.24 NS	524.1	6.86 **	0.846	1.36 NS	18.62	5.46 **	3.65	1.83 NS
Oestrone Levels	2	43.67	39.03 **	126.1	<1	0.180	<1	3.87	1.13 NS	0.60	<1
Treated v. Untreated	1	53.17	47.52 **								
Dose 0.003 v. 0.006 ug.	1	34.17	30.54 **								
Cortisol Levels	3	2.67	1.12 NS	2210.4	10.70 **	2.947	4.75 **	2.35	<1	27.65	13.87 **
Treated v. Untreated	1			5031.5	24.35 **	6.510	10.48 **			59.32	29.76 **
Dose 12.5 v. 25.0 ug.	1			24.2	<1	2.250	3.62 +			0.49	1
Dose 50.0 v. 12.5 + 25.0 ug.	1			1575.5	7.63 *	0.083	<1			23.14	11.61 **
Blocks x Oestrone Levels	10	1.60	1.43 NS	65.2	<1	0.293	<1	2.52	<1	1.25	<1
Blocks x Cortisol Levels	15	2.39	2.13 *	113.9	1.49 NS	0.395	<1	1.23	<1	1.19	<1
Oestrone Levels x Cortisol Levels	6	1.01	<1	206.6	2.71 *	0.153	<1	1.35	<1	0.59	<1
Blocks x Oestrone x Cortisol Levels	29[1.]	1.12		76.4		0.621		3.41		1.99	

** Highly significant ($p < 0.01$). * Significant ($p < 0.05$ but > 0.01). + Not significant ($p > 0.05$ but < 0.10). NS: Not significant ($p > 0.05$).

1. Residual degrees of freedom reduced by one to compensate for a missing plot. 2. Counted on the first and second pairs of thoracic glands.