animals begin to die in the older age groups of the back-cross it will tend to lower the final percentage of tumors in these generations. If the present high tumor incidence of mammary tumors in Cross II persists it might be considered the result of chromosomal influences from the dilute brown chromosomes. Cross IV, however, should be actually no greater than on the F_1 and F_2 generations above mentioned. If, therefore, chromosomal influences are operative to any measurable degree Cross IV should eventually show no higher incidence of mammary tumors than 40 per cent., while Cross II should remain appreciably higher than that figure. Further data are needed to settle this point.

The relative age of the populations in the four crosses is a factor to be considered. The mean age in days of the four is as follows: I, 499; II, 444; III, 439; IV, 431. It will be noted that such difference as exists has resulted in as good or better chance for the crosses showing no mammary tumors as for those showing them.

While the incidence of mammary tumors has shown a clearly "extra-chromosomal" type of distribution, the two pairs of mendelian color factors B black—b non-black (brown), and D intense—d, dilute have segregated normally. The actual count in the two crosses where such segregation was discernible is given in Table 2.

TABLE 2

	Black	Dilute black	Brown	Dilute brown	Total
I					
Observed	170	162	134	172	636
Expected	159	159	159	159	638
II					
Observed	163	133	128	152	576
Expected	144	144	144	144	576

The experiments are being continued and will be fully discussed on their completion. It should be clearly understood that larger numbers of animals may modify somewhat the quantitative values of the various groups. On the other hand, there is little doubt that the major difference between Groups II and IV, on one hand, and I and III, on the other, will persist.

Although the numbers of mice so far autopsied in Crosses I and III are small, the absence of mammary tumors is interesting and may, if it persists, have considerable significance. The fact that the completed series of F_1 and F_2 animals, to which reference has been made, showed in all crosses some mammary tumors remains to be explained by further experimentation. Using the symbols already employed, the F_1 and F_2 generations may be tabulated as follows:

dBF_1 ♀ $\frac{CE}{C} \times$ ♂ $\frac{c}{c}$ = 39.82 per cent. mammary tumors in 113 mice

BdF_1 ♀ $\frac{ce}{c} \times$ ♂ $\frac{C}{C}$ = 6.06 per cent. mammary tumors in 379 mice

dBF_2 ♀ $\frac{CE}{c} \times$ ♂ $\frac{C}{c}$ = 35.29 per cent. mammary tumors in 664 mice

BdF_2 ♀ $\frac{ce}{C} \times$ ♂ $\frac{C}{c}$ = 5.96 per cent. mammary tumors in 688 mice

We may thus conclude that in the four types of back-cross cited, the incidence of mammary tumors in mice depends primarily upon the direct transmission of extra-chromosomal influences. This confirms completely the earlier experiments with F_1 and F_2 mice.

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ADRENAL INSUFFICIENCY IN THE MARMOT AND OPOSSUM AND THEORIES OF CORTICO-ADRENAL FUNCTION

COMMON laboratory animals (cat, dog, rat, rabbit, guinea-pig) which have been adrenalectomized show diminutions in serum sodium and chloride content, and Addisonian patients in crisis are apparently similarly affected.[1, 2, 3, 4] It has also been demonstrated that an increased renal excretion of sodium and chloride is either the result or the cause of the subnormal values found in blood serum and tissues after adrenal removal. If compensation for excessive loss of sodium chloride is made by the injection or oral administration of saline, adrenal insufficiency is said to be relieved[2, 3, 5]; again, if the sodium content of the body is reduced experimentally, a condition resembling that of adrenal insufficiency is said to result.[7]

On the basis of these observations several experimenters have expressed the belief that the adrenal cortex is intimately concerned with sodium chloride

[1] H. Silvette and S. W. Britton, *Amer. Jour. Physiol.*, 104, 399, 1933; *ibid.*, 108, 535, 1934; *ibid.*, in press; also unpublished results.

[2] R. F. Loeb *et al.*, SCIENCE, 76: 420, 1932; *Proc. Soc. Exp. Biol. Med.*, 30: 808, 1933; *Jour. Exp. Med.*, 57: 775, 1933.

[3] G. A. Harrop *et al.*, *Jour. Amer. Med. Assoc.*, 100: 1850, 1933; *Jour. Exp. Med.*, 57: 305; *ibid.*, 58: 1, 17.

[4] W. W. Swingle *et al.*, *Amer. Jour. Physiol.*, 107: 259, 1934.

[5] J. M. Rogoff, *Jour. Amer. Med. Assoc.*, 103: 1764, 1934.

[7] A. Gilman, *Amer. Jour. Physiol.*, 108: 662, 1934.

metabolism, and that death after adrenalectomy probably occurs because of reductions in sodium and chloride concentration and correlated derangements of blood volume and tissue fluids.[2, 3, 4]

It appeared significant indeed that all the animal types which have been investigated showed reductions in sodium and chloride values after adrenal removal. Diminutions in blood sugar and liver and muscle glycogen were moreover concurrently observed.[1, 8] Blood sugar and liver glycogen values were reduced in many cases, indeed, to the point of exhaustion. Several years ago one of us[6] pointed out that the adrenal cortex appeared to have its main action on carbohydrate metabolism, and recent results have confirmed this belief.[8] The challenge to our carbohydrate theory of cortico-adrenal function by what may be termed the sodium chloride theory has led us to investigate the issues further.

In this connection we have for some time entertained the possibility of finding an animal species in which the two groups of phenomena, *i.e.*, the carbohydrate and sodium chloride changes, might perhaps be separated by nature—an animal type in which, for example, the carbohydrate levels might become reduced following adrenalectomy while the salt content might remain normal, or *vice versa*. In such case the primary responsibility for the adrenalectomized animal's death might be directly indicated.

Functional conditions in the opossum (*Didelphys virginiana*) and the marmot (*Arctomys monax*), we have now discovered, offer a key to the solution of the problem. Following adrenalectomy these animals progressively lose weight, show anorexia and all the general symptoms of insufficiency, and eventually die in typical hypoglycemic convulsions. If such animals are sacrificed when insufficiency symptoms appear the blood sugar is found to be very low, and muscle and liver glycogen are profoundly decreased as in the case of the commoner laboratory animals. Serum sodium and chloride values show a definite *increase*, however, over the normal controls, and the sodium chloride content of the muscles also appears to be increased. The muscle water percentage was observed, furthermore, to be significantly reduced. Sodium chloride and water balance are therefore strikingly different in the marmot and the opossum from any other species which have been investigated to date.

Analyses of the daily urinary output of opossums before and after adrenalectomy are in correlation with the above observations: daily excretion of sodium chloride is considerably *less* after operation than before.

[8] S. W. Britton and H. Silvette, *Amer. Jour. Physiol.*, 100: 693, 701, 1932; SCIENCE, 77: 366, 1933; *Amer. Jour. Physiol.*, 107: 190, 1934.

[6] S. W. Britton, *Physiol. Rev.*, 10: 617, 1930.

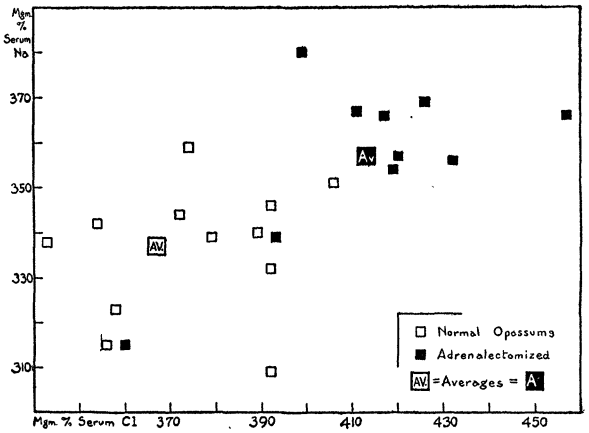

FIG. 1. Serum sodium and chloride levels in normal and adrenalectomized opossums.

In other experimental animal types, sodium chloride elimination is in contrast greater after adrenal excision.[1, 2, 3, 4, 9] The graph herewith depicts the sharp contrast in the opossum in serum sodium and chloride levels before and after adrenal ablation.

The opossum, it is appreciated, represents one of the lowliest of mammalian types, and it has been said that the marmot is a very ancient form. Marsupial nursing and hibernating activities are undoubtedly to be listed among the more primitive functional characteristics of mammals. It is nevertheless hardly conceivable that the internal secretory mechanisms of these animals should differ in any notable degree from those observed in the case of other laboratory animals. Although not categorical in their expressions, hormones do appear to exert their influence with duplicable uniformity through different animal species. Thus, if sodium chloride metabolism were controlled through cortico-adrenal function in one animal, it would probably be regulated similarly in others.

The opossum and the marmot apparently metabolize carbohydrates in essentially the same manner as higher mammalian types. Salt and water balance after adrenal excision, however, is not at all similar to that found in other adrenalectomized animals. The evidence indicates that the adrenal cortex is not specifically involved in the control of sodium and chlorides in the organism. It appears singular that adrenalectomy should lead to opposite effects on sodium chloride in different animals. The possibility that cortico-adrenal activities are related to kidney function may be entertained. It is perhaps more significant, however, that in their carbohydrate economy the Virginian marsupial and the marmot run true to higher forms.

It is concluded that the adrenalectomized opossum and marmot die from carbohydrate insufficiency and

[9] M. I. Rubin and E. T. Kricke, *Proc. Soc. Exp. Biol. Med.*, 31: 228, 1933.

not from sodium chloride disturbance. The results appear to refute the theory that sodium chloride metabolism is specially regulated by the adrenal cortex. They furnish further strong evidence, however, in favor of our first-proposed carbohydrate theory of cortico-adrenal function.

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THE PERIPHERAL ACTION OF BARBITURATES[1]

LIEB and Mulinos[2] and other authors[3,4] described the action of amytal in impairing vagus cardiac inhibitory effects. According to Swanson and Shonle,[5] this action was regarded as characteristic for amytal, since a closely related barbiturate, iso-amytal (1-methyl-butyl ethyl barbituric acid or pentobarbital), failed to produce a similar peripheral depression of the cardiac vagus in doses of 5 to 20 mgm per kgm.

In a large series of experiments, using dogs and rabbits, we confirmed the original observation of Lieb and Mulinos with amytal. We found that amytal in doses ranging from 50 to 60 mgm per kgm abolished the typical cardiac vagus effect, *viz.*, the cardiac inhibition which resulted from stimulation of the peripheral vagus. Experiments of a similar nature revealed the fact that this peripheral action of amytal is also exhibited after very large doses of pentobarbital-sodium and other barbiturates. The animals used in these experiments were kept alive with picrotoxin. Doses of pentobarbital-sodium varying from 40 to 100 mgm and barbital-sodium in doses of 500 to 1,100 mgm per kgm completely abolished the typical vagus response to faradic stimulation. Phenobarbital-sodium in doses of 100 to 350 mgm per kgm impaired but did not abolish the cardiac vagus effect. In all these instances where the peripheral vagus effect had been abolished by barbiturates, the central vagus effects, *e.g.*, the respiratory inhibition following the faradic stimulation of the central end, remained unimpaired.

The mechanism of the depression of the cardiac vagus function by barbiturates was disclosed by another series of experiments. The following facts relative to this problem have been ascertained:

(a) Pilocarpine (1 mgm per kgm) slowed the heart of barbiturate-treated animals which did not respond to peripheral vagus stimulation.

(b) Large doses of acetyl choline (0.1 mgm per kgm) produce vasodilation and cardiac inhibition in the animals which had received barbiturates.

(c) Doses of physostigmine (0.2 to 0.35 mgm per kgm) which were insufficient to produce slowing of the heart rendered the cardiac vagus sensitive to faradic stimulation. Also large doses of acetyl choline had the same effect after the blood pressure and heart rate returned to the original level.

These experiments suggest that the cardiac vagus impairing action of barbiturates is not an atropine-like action but probably a nicotine-like effect on the vagus ganglia.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE TYPE OF ERGOMETER

IN order that the greatest value may be obtained from many laboratory experiments in human physiology it is desirable that students, particularly those in the preclinical years of medicine, understand the relationship between basal manifestations and known amounts of activity. Ergometers, when constructed to give a reasonably accurate measure of the work performed, are usually so costly that one piece of apparatus is to be found in a laboratory. Hence experiments must be either done by very large groups or the experiment conducted as a demonstration.

It has been the policy in the author's laboratory to correlate to a maximal degree the effect of known amounts of activity with the various physiological systems. In order to do this a simple yet efficient type of ergometer has been devised of such a nature that each table of a special laboratory devoted to human physiology is equipped with this device. The arrangement has proven of great convenience, since students have an opportunity to evaluate the effect of graded activity upon such physiological manifestations as blood pressure, pulse rate, metabolism and respiration. A further definite advantage of the apparatus rests in the fact that preliminary measurements can be taken under nearly basal conditions, which is not true of many common forms of ergometer.

The ergometer is constructed at one end of a table, 72×30 inches, with legs of sufficient height that the usual type of clinical metabolism apparatus may be

[1] From the Department of Pharmacology and Materia Medica, Georgetown University, School of Medicine, Washington, D. C.

[2] Lieb and Mulinos, *Proc. Soc. Exper. Biol. and Med.*, 26: 709, 1929.

[3] Shafer, Underwood and Gaynor, *Am. Jour. Physiol.*, 91: 461, 1930.

[4] Garry, *Jour. Pharmacol. Exper. Therap.*, 39: 129, 1930.

[5] Swanson and Shonle, *Jour. Lab. and Clin. Med.*, 16: 1056, 1931.