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ADRENAL INSUFFICIENCY IN AMERICAN MONKEYS[1]

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While numerous experiments on adrenal function have been carried out on lower mammals and even on sub-mammalian forms, the primates have hardly been considered at all in this field of study. It would appear, however, that observations on adrenal insufficiency in monkeys might add more particularly to our knowledge of human adrenal physiology, and also shed fundamental light on the clinical condition of Addison's disease in man. Opportunity was recently afforded for studying, in their native habitat, three common species of American monkeys, viz., the capuchin or white-faced monkey, *Cebus capucinus imitator* Thomas; the night-monkey, *Aotus zonalis* Goldman; and the marmoset or squirrel-monkey, *Leontocebus geoffroyi* Pucheran. In these animals observations on adrenal insufficiency proved to be particularly interesting.

METHODS. The animals used were kept in large open-air cages, and frequently other monkeys from the adjacent jungle (Barro Colorado Island) came by and chattered with the experimental group. A complete diet of mixed tropical fruits, with occasional small amounts of meat relished by these monkeys, was given. Adult or near-adult males and females were used. The adrenals were easily accessible and were removed with very little trauma in one operation under ether, lasting only 5 to 10 minutes. No sterile precautions were used in most cases, since it was found that ordinary cleanliness alone gave good (non-infective) operative results in work in the tropics. Normal and operated animals were utilized in the non-fasting state.

Protein-free filtrates of heparinized plasma were prepared by adding 3 volumes of distilled water and 1 volume of 20 per cent trichloracetic acid to 1 volume of plasma. After standing and filtration the trichloracetic acid filtrates were stored in glass-stoppered Pyrex bottles. Sodium

[1] The physiological phases of these studies were carried out at the Barro Colorado Island Laboratory, C. Z., and the Gorgas Memorial Laboratory, Rep. Panama, under the terms of a John Simon Guggenheim Memorial Fellowship held by the senior author during 1937–1938. The thanks of the authors are gratefully extended to Mr. James Zetek and Dr. Herbert C. Clark of these laboratories, respectively, for their unfailing courtesies during the investigations.

[2] E. R. Squibb and Sons Fellow in Physiology.

(Butler and Tuthill, 1931) and potassium (Kramer and Gittelman, 1926) analyses were made on 5 cc. quantities of plasma filtrate evaporated to dryness with a drop of nitric acid and redissolved in 1 cc. of distilled water. Chloride and urea determinations were made directly on the trichloracetic acid filtrates, the former by the method of Van Slyke and Sendroy (1923) and the latter by the gasometric procedure of Van Slyke and Kugel (1933). Plasma glucose determinations were made by the method of Folin and Malmros (1929) on carefully neutralized aliquots of the protein-free filtrates.

Portions of muscle tissue weighing about 1 gram were placed in previously weighed small tubes containing 4 per cent formaldehyde. Later, the tubes were again weighed to give by difference the weight of fresh tissue, and the contents poured into aluminum weighing-pans of known weight and placed in an oven at 105° until constant weight was attained. The added formaldehyde solution evaporated off completely, as did the muscle water, and the true water content of the muscle was thus determined.

Tissue glycogen determinations were made by the Pflüger procedure modified for small amounts of tissue (Silvette and Britton, 1932). Control determinations showed that after solution of the tissue had taken place in the hot alkali, no glycogen loss occurred even though the alkaline glycogen mixture stood for several weeks.

Results. Exceedingly high liver glycogen levels, even for animals which were digesting food, were commonly found in the normal monkeys used. Several cases varied from 8 to almost 12 per cent (table 1). The averages were: capuchins 2.8, night-monkeys 6.6, and marmosets 2.4 per cent. In this connection it was found that at the time of sampling, the liver tissues were strikingly glutinous to touch. Plasma glucose and muscle and heart glycogen levels in normal monkeys did not differ greatly from those found in man and many other animals. Similarly, serum sodium and chloride and muscle water were comparable.

Within a short while after adrenal removal, food was taken readily and the usual playful and other responses appeared normal. After a few days or a week or so, insufficiency symptoms of lethargy and weakness began to appear, and continued to progress gradually for one or two days. Even up to the time and during the period of prostration, however, food—especially bananas—would often be taken. Convulsions invariably supervened and were of the strikingly typical hypoglycemic type—tonicoclonic spasms, clawing, turning, limb extension, dorsiflexion, etc. They frequently did not appear until an animal had been prostrated for several hours. At first they were observed about once an hour or so, and then gradually became more frequent until after about six hours they occurred once in 15 to 20 minutes. Convulsive cries and increased salivation were

TABLE 1

Carbohydrate, salt and other blood and tissue levels in normal and adrenalectomized new-world monkeys

ANIMAL NUMBER	SEX	EXPERIMENTAL CONDITION	SURVIVAL	SERUM				GLYCOGEN			MUSCLE WATER
				Sodium	Chlorides	Urea	Sugar	Liver	Muscle	Heart	
White-faced monkey (*Cebus capucinus imitator* Thomas)											
			hours	*mE per l.*	*mE per l.*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	F	Normal		140.4	110.4	88	114	1.08	0.51	0.56	73.2
2	F	Normal		143.8	114.6	65	89	1.81	0.72	0.60	75.2
3	F	Normal		150.1	117.2	52	133	1.46	0.61	0.48	72.9
4	F	Normal		139.1	115.0	54	69	0.57	0.69	0.60	74.9
5	F	Normal		136.5	110.2	139	111	9.55	0.42	0.36	76.5
6	F	Normal		142.4	122.8	126	109	1.52	0.78	0.47	74.1
7	F	Normal		150.8	112.8	142	117	1.32	0.75	0.34	74.9
11	M	Adrex†	187	124.2	98.6	132	21	0.13	0.51	0.22	76.1
12	M	Adrex	48	122.9	106.0	142	18	0.07	0.48	0.18	76.2
13	M	Adrex	126	125.5	104.6	103	32	0.10	0.41	0.20	76.2
14	M	Adrex	437	126.8	103.2	175	30	0.12	0.20	0.02	75.0
15	F	Adrex	25	130.7	110.6	156	33	0.21	0.56	0.14	76.0
Night monkey (*Aotus zonalis* Goldman)											
1	F	Normal		122.2	99.6	118	144	1.41	0.61	0.16	73.6
2	F	Normal		137.2	106.6	130	114	2.75	0.42	0.26	74.6
3	F	Normal		139.1	112.4	127	100	8.75	0.69	0.25	74.9
4	F	Normal		147.2		88	122	3.34			75.4
5	M	Normal		144.3	113.8	87	111	11.8	1.27	0.32	74.4
6	F	Normal		146.3	117.2	84	125	11.6	1.27	0.36	75.0
11	M	Adrex	160	126.9		118	64	0.13	0.29	0.19	72.3
12	M	Adrex	194	133.9		208	46	0.09	0.35	0.27	74.2
13	F	Adrex	21	137.8	98.8	165	163*	0.22	0.42	0.30	73.5
14	M	Adrex	78	134.6		91	127*	0.18	0.32	0.08	68.8
Marmoset (*Leontocebus geoffroyi* Pucheran)											
1	F	Normal		158.8	113.2	113	109	3.77	0.52	0.18	71.9
2	F	Normal		143.7		86	80	1.71	0.15	0.33	73.8
3	M	Normal		158.0	120.6	109	106	1.61	0.96	0.46	76.3
11	M	Adrex	63					0.21	0.25	0.09	75.5
12	M	Adrex	69	136.5		191	31	0.13	0.44	0.25	74.1
13	M	Adrex	27	150.2	112.4	179	29	0.19	0.31	0.24	74.8

* These high figures are probably explained by the very short survivals after operation.

† Adrenalectomized.

observed on several occasions. In the last stages of insufficiency the seizures often tended to become continuous, and sometimes ended in convulsions of an obviously different, asphyxial character. Many of the adrenalectomized animals were sampled shortly after convulsions had set in; in a few cases they were utilized at the point of death.

Extremely low serum sugar and liver glycogen values were found in monkeys suffering from adrenal insufficiency. Considering all series (see footnote exception, table 1), the average reduction of serum sugar from normal was over 70 per cent (9 cases), while liver glycogen was reduced over 90 per cent (12 cases). Muscle and heart glycogen values were also greatly reduced following adrenalectomy (see averages, fig. 1).

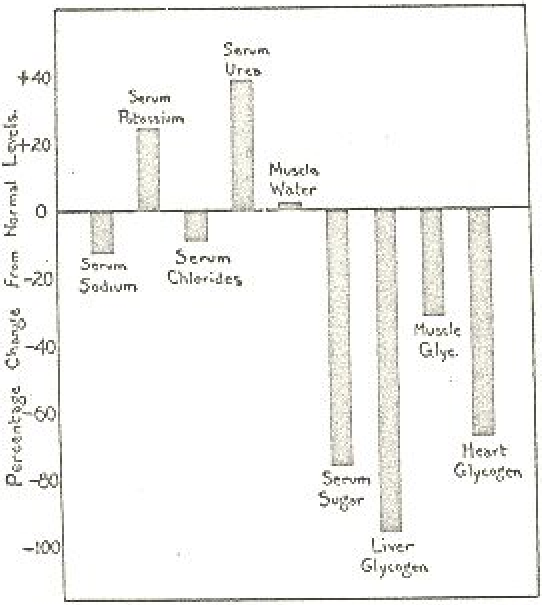

Fig. 1. Blood and tissue changes in the white-faced monkey after adrenalectomy

It is worthy of note, however, that food was often found in the stomach, and food residues almost invariably in the small intestines, in the adrenalectomized monkey at autopsy.

Decreases in serum sodium and chlorides, and increases in serum urea and potassium, were observed in adrenal insufficiency in monkeys, in keeping with data derived from other animal types. Also, muscle water usually tended to show an increase.

The survival periods after adrenalectomy were variable, and ranged from 1–18 days in capuchins (average 7), 1 to 8 days in night-monkeys (average 5), and 1 to 3 days in marmosets (average 2).

Discussion. The small amount of adrenal work hitherto carried out on the monkey has usually been hidden in reports on other animal forms. Kahn (1912) kept two *macacus rhesus* monkeys alive each for 5 days after adrenal removal, and in gross determinations found only traces of glycogen present in the liver after adrenal insufficiency had set in. Apparently there is some difficulty in maintaining adrenalectomized monkeys alive with cortico-adrenal extract. Hartman and Winter (1933) have reported a series of four animals (macaques) which lived 4, 16, 26 and 35 days after operation with the aid of "cortin." Activity and infection were found to bring about marked exhaustion from which resuscitation could not be effected even with large doses of extract. Harrop and Weinstein (1933) kept two macaques alive on extract 11 and 42 days after adrenalectomy, and noted skin pigmentation in the longer-lived case. Firor and Grollman (1933) state that they made observations on 6 monkeys, but give no survival periods or other data.

Allen and his colleagues (1937) kept two adrenalectomized-ovariectomized monkeys alive for short periods with cortico-adrenal extract. One partially adrenalectomized macaque survived for a few weeks and another for a few months in Stewart and Rogoff's laboratory (1919).

No quantitative blood-chemical or tissue analyses were mentioned in the above brief reports, and only a few individuals were considered. The present work covers in monkeys the more important changes such as are known to occur in adrenal insufficiency in lower animal types. It is apparent that blood and tissues are affected similarly as far as direction of change is concerned. All the New-World monkeys examined, however, appear to suffer much more profoundly than other animals from carbohydrate deficiencies following adrenal removal. Almost certainly the figures for liver glycogen and serum sugar indicate complete exhaustion in these depots. They are in sharp contrast to the high levels found in the unoperated animals. It should be emphasized, too, that food was taken fairly readily by the monkey when prostrated from adrenal insufficiency, and food residues were usually found in the gastro-intestinal tract at autopsy. Besides the liver and blood changes, muscle and heart glycogen reductions were also severe.

The hypoglycemic convulsions, which persisted for several hours preceding death from adrenal loss, were in correlation with the carbohydrate losses. We have studied to date many animal series covering more than a dozen different mammalian species suffering from adrenal insufficiency, and in no other animal have the convulsive seizures been so severe and prolonged, and the carbohydrate reserves so low, as in the present series of monkeys. It is clear that these primates from which the adrenals have been removed suffer most and die chiefly from carbohydrate deficiencies.

In a recent paper Grollman (1938) reports that the cortico-adrenal hormone is well able to maintain normal carbohydrate levels in adrenalectomized or adrenalectomized-pancreatectomized rats. He was unable to influence blood sugar in normal and in hypophysectomized rats with cortico-adrenal extracts, however, and hence did not consider that the adrenal cortex was preëminently involved in regulating carbohydrate metabolism. Grollman, it should be emphasized, used only very small amounts of extract—1 cc. or less—in rats which are notoriously resistant to hormones. In earlier papers we have already demonstrated the positive action of our adrenal extracts on carbohydrate levels, however, in both normal and hypophysectomized animals (Britton and Silvette, 1932; Corey, 1938). The present and other recent experiments (Britton and Silvette, 1937; Britton, Silvette and Kline, 1938) have further shown the revolutionary effects of adrenal removal on carbohydrate metabolism in a wide variety of animal types.

SUMMARY

New-World monkeys—capuchins, night-monkeys, marmosets—show tissue and blood chemical changes after adrenal removal which are rather similar to those found in other higher mammalian forms. Serum sodium and chlorides are significantly reduced while muscle water tends to be increased. Serum potassium and urea are raised.

Extreme carbohydrate changes, more profound than those which occur in other mammalian types, were observed. In 9 cases serum glucose reductions in adrenal insufficiency averaged over 70 per cent, and in 12 cases liver glycogen values were reduced over 90 per cent (table 1). These figures appear to represent exhaustion of available carbohydrates in these depots. In muscle and cardiac tissues glycogen levels were also significantly reduced.

The very low carbohydrate levels in adrenal insufficiency contrast with markedly high liver glycogen values, which may approximate 12 per cent, found in normal monkeys.

Hypoglycemic convulsions are a prominent feature of adrenal insufficiency in monkeys. Usually they last over a period of several hours and are characterized by very severe spasms, eventually giving place at the last to seizures of an asphyxial type.

Evidently the highly important function of the liver as a former and furnisher of glycogen is rapidly abolished in the absence of the cortico-adrenal hormone. Further, there appears no doubt that the first cause of death in adrenal insufficiency in the primates examined is the exhaustion of carbohydrates in blood and hepatic tissues.

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