CXXXIII. KETOSIS IN THE HYPOPHYSECTOMIZED RAT

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It is generally agreed that the injection of anterior pituitary extracts will increase ketosis in animals. Burn & Ling [1930] originally demonstrated that these extracts increased ketonuria in rats fed on fat and later Anselmino & Hoffmann [1931] showed that there was an increase in blood acetone after injection of similar extracts. These results have been amply confirmed by others and have led to the suggestion that there is a factor present in the anterior pituitary which plays some part in fat metabolism. It has not yet been decided, however, whether the effects that are produced are due to a specific factor for fat metabolism, as suggested by Magistris [1932] and by Anselmino & Hoffmann [1934], or whether they may be the result of one or more of the other known factors in the anterior pituitary, as has been suggested by Shipley & Long [1938].

Since anterior pituitary extracts increase ketosis, it would be expected that hypophysectomy would cause a reduction in ketonuria and a decrease in the blood acetone bodies. Rietti [1933] has shown that the ketonuria in dogs which have been rendered diabetic either by pancreatectomy or by the administration of phlorhizin is less in hypophysectomized animals than in those with the pituitary intact.

The purpose of the present work was to investigate the effects of hypophysectomy on the ketosis produced in rats by a diet of fat.

Methods

The animals used were young females of the London strain of Wistar rat. Females were chosen because they are known to be more susceptible than male animals to ketosis. Rats weighing 120–160 g. were hypophysectomized under ether anaesthesia by the technique of Smith [1927], slightly modified. At 10–20 days after operation they were placed in metabolism cages, fed on a diet consisting of butter with the addition of a 3% salt mixture, as described by Wigglesworth [1924], and their urine was collected in 24-hr. samples under toluene. The glass collecting vessels were washed with distilled water and the urine and washings made up to a volume of 20–25 ml. At the completion of the experiment, the animals were bled by heart puncture under chloroform anaesthesia. Control animals received similar treatment. The total acetone bodies were determined in blood and urine by Van Slyke's gravimetric method with Denigès' reagent [Peters & Van Slyke, 1932], modified for the smaller quantities of material, i.e. 10 ml. urine and 2 ml. blood.

The hypophysectomized animals were very sensitive to cold and in some of the earlier experiments, when the metabolism cages were in a laboratory the temperature of which was not controlled, the animals became cold and moribund

after a few days on the fat diet. Therefore, both hypophysectomized and control animals were kept in the animal room at a temperature of 75° F., which was controlled by a thermostat.

Results

Ketonuria only was investigated in our first group of experiments. In normal rats on the fat diet there was a variable but definite excretion of acetone bodies, but in the hypophysectomized animals on the same diet there was never more than a trace of ketonuria. It had been shown previously [Anderson & Anderson, 1927] that the injection of adrenaline caused an increase in the ketonuria of young rats fed on a fat diet. Therefore, adrenaline was administered subcutaneously to the control and experimental animals in doses of 0.15 mg. per rat on the 4th or 5th day of fat feeding. Our normal animals showed a definite increase in ketonuria after the injection of adrenaline, but the hypophysectomized animals showed no increase whatever.

In four hypophysectomized rats and their controls the blood acetone bodies were estimated at the end of the experiment. Three of these are shown in Figs. 1-3.

In Fig. 1, the daily excretion of total acetone bodies in the urine is plotted for a hypophysectomized rat and its control, and the levels of blood acetone bodies at the end of the experiment are shown by the vertical columns. It will be seen that while the control rat showed a considerable ketonuria, starting on the 3rd day and rising to 65 mg. on the 4th, the hypophysectomized rat did not excrete more than 3 mg. even after 5 days on the fat diet. By contrast, the level of acetone bodies in the blood at the end of the 5th day was nearly the same in both animals, namely, 55 mg./100 ml. in the hypophysectomized animal, as compared with 54 mg. in the control. Another hypophysectomized rat, which was maintained on a fat diet for 7 days, did not excrete any appreciable amount of acetone bodies in the urine, and on the 7th day blood acetone bodies were 25 mg./100 ml.

The results after adrenaline are shown in Figs. 2 and 3. In Fig. 2 the control animal shows a large excretion of acetone bodies, rising to 78 mg. after the injection of adrenaline, while the hypophysectomized animal excreted only traces, i.e. less than 1 mg. per day. The level of acetone bodies in the blood at the end of the 5th day was high in both rats, but higher in the hypophysectomized rat than in the control—88 mg./100 ml. as compared with 63 mg. In the other experiment shown (Fig. 3), in which adrenaline was injected on the 6th day, the control animal showed a slight ketonuria rising to 19 mg. on the 3rd day, falling to 3 and 4 mg. on the 4th and 5th days respectively and rising to 20 mg. after adrenaline, while the hypophysectomized rat excreted only traces. Here again both animals showed a ketonaemia, though of less degree, and the level in the hypophysectomized rat (45 mg.) was greater than that in its control (23 mg.).

It is of interest to note that after the injection of adrenaline glycosuria occurred in the controls, but not in the hypophysectomized rats.

The hypophysectomized rats all had hypoglycaemia at the end of the experiment, with blood sugar levels of 30-40 mg./100 ml., while the control animals showed a less severe reduction in blood sugar, which varied between 42 and 67 mg./100 ml.

DISCUSSION

Our results show that the hypophysectomized rats had no appreciable ketonuria after being fed on a diet of pure fat for as long as a week, whereas the control animals excreted variable but definite amounts of acetone bodies. These

Fig. 1. Abscissa=days. Ordinate=mg. acetone bodies (acetone + acetoacetic + β -hydroxybutyric). Broken line *H*=urinary excretion by hypophysectomized rat. Continuous line *C*=urinary excretion by control. Black vertical column *H*=blood acetone bodies mg./100 ml. in the hypophysectomized rat. White column *C*=blood acetone bodies in the control.

Fig. 2. Abscissa, ordinate etc. as in Fig. 1.

Fig. 3. Abscissa, ordinate etc. as in Fig. 1.

results agree with those of Rietti [1933], referred to above. The subcutaneous injection of adrenaline produced a considerable increase in the ketonuria of the normal rats, as in the previous experiments reported by Anderson & Anderson [1927]. In our hypophysectomized rats, however, the injection of adrenaline had no apparent effect on the excretion of acetone bodies. It should be noted that Russell & Cori [1937] reported that the effect of subcutaneous injection of adrenaline on carbohydrate metabolism was less in the hypophysectomized than in the normal rat, whereas intravenous injection produced the same effect in both.

The absence of ketonuria in the hypophysectomized animals might be explained by the removal of a "ketogenic factor" in the anterior pituitary. The blood acetone bodies, however, instead of being decreased, were actually higher than in the corresponding control animals, whether adrenaline had been administered or not. These results are not necessarily contradictory to the hypothesis of a pituitary "ketogenic factor", because the high level of blood acetone might not have been reached had even small amounts of acetone been excreted in the urine.

The apparent inability to excrete acetone bodies after hypophysectomy must be due to some alteration in renal function, either to a general failure, or to a specific change in the threshold for these bodies. In the dog, hypophysectomy appears to produce a temporary decrease in the concentrating power of the kidney. White & Heinbecker [1938] reported that in hypophysectomized dogs there was a rise in blood urea from 20 to 40 mg./100 ml. at 20 days after the operation, followed by a fall towards normal, and that the urea and creatinine clearances were decreased by nearly half at first, then rose towards normal after a few weeks. Their operations were performed by the ventral approach through the palate, thus avoiding damage to the hypothalamus. In this respect their operations were similar to our own in which damage to the hypothalamic region was excluded.

We have no evidence as to whether a fall in the concentrating power of the kidney for urea etc. occurs in rats after hypophysectomy. Urinary output was not measured in our experiments, but there was no obvious difference in the urinary output of the hypophysectomized and control animals. In a previous publication we reported that the blood inorganic phosphate of the rat was considerably decreased after removal of the pituitary [Anderson & Oastler, 1938]; this decreased concentration of phosphate would not be expected if there were a severe degree of impairment of renal function.

In our opinion, a failure in kidney function of the order found by White & Heinbecker [1938] is not sufficient to account for the almost complete failure of the hypophysectomized rat to excrete acetone bodies.

Shipley & Long [1938] have shown that the urinary excretion of acetone bodies in rats does not rise above 5 mg. in 24 hr. until the blood level has reached a value of about 25 to 30 mg./100 ml. Beyond this point they found that the excretion rose sharply. They consider that this represents a threshold level for β -hydroxybutyric acid and acetoacetic acid; acetone had already been shown not to be a threshold substance [Widmark, 1920; Briggs & Shaffer, 1921]. In our experiments, the normal animals showed the usual marked variation in the degree of ketonuria which seems to depend on the degree of ketonaemia. In two of the normal animals (Figs. 1, 2), there was a great excretion of acetone bodies and in them the blood acetone was raised far above the threshold value. In the animal with a smaller degree of ketonuria (Fig. 3), the blood acetone was at the threshold value. Some of our other normal animals settled down to an excretion

of only a few mg. per day, and in the only one of these in which blood acetone bodies were estimated, only traces were found. In the hypophysectomized animals the threshold value was exceeded in each case, and yet there was no appreciable ketonuria. Russell & Cori [1937] showed that the threshold for sugar was raised in rats after hypophysectomy. They demonstrated this by the administration of adrenaline intravenously and by the infusion of glucose. They concluded that the excretion of sugar in the urine was not a good index of the adrenaline action because of the alteration of kidney function in the hypophysectomized rat, whereby, although the blood sugar was higher than in the controls, the excretion was considerably lessened.

Neufeld & Collip [1938] observed that in fasting adrenalectomized rats treated with potent ketogenic pituitary extracts there was no ketonuria, but that there was a ketonaemia equal to that of normal rats similarly treated, provided that the animals were in good condition; they suggested that the failure of the adrenalectomized rats to show ketonuria was due to a raised kidney threshold for acetone bodies.

The results of our experiments would suggest that the threshold for acetone bodies is also raised after hypophysectomy. Whether this is directly due to lack of the pituitary, or can be accounted for by atrophy of the adrenal cortex from lack of the adrenotrophic factor cannot be decided on the evidence available.

Our results prove that the presence or absence of ketonuria cannot be accepted as an indication of the degree of ketosis in the hypophysectomized rat.

SUMMARY

1. Hypophysectomized female rats excreted only traces of acetone bodies in the urine when fed on a diet of fat for as long as a week, nor did they show any increase in ketonuria after the subcutaneous injection of adrenaline. Control animals showed a variable but definite ketonuria, which was increased by the injection of adrenaline.

2. At the end of the period of fat-feeding, blood acetone bodies were higher in the hypophysectomized than in the control rats and were above the normal threshold level.

3. It is suggested that the absence of ketonuria in the hypophysectomized rats is due to an increase in the renal threshold for acetone bodies after the removal of the pituitary.

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