CLXXXIII. THE EFFECT OF ADRENALINE ON KETOSIS IN PHLORIDZINISED AND NORMAL RATS.

By ALAN BRUCE ANDERSON
AND MARGARET DAMPIER ANDERSON.

From the Biochemical Laboratory, Cambridge.

(Received October 31st, 1927.)

IN the course of metabolic experiments on phloridzinised rats it was observed that doses of adrenaline increased the excretion of acetone bodies. This paper gives an account of some experiments undertaken in an attempt to investigate this increase and a discussion of its significance in relation to other published observations on the action of adrenaline.

Rats were phloridzinised by Coolen's method, receiving 50 mg. phloridzin in oil every day or every two days. Urine was collected in 24-hour samples in the usual way. Acetone bodies were estimated by the method used by Wigglesworth [1924]. A satisfactory diet for the fully phloridzinised rat had been found in previous work to consist of caseinogen 75 parts, fat 20, salts 5, agar-agar 5. The salt mixture was that employed by Wigglesworth ($\text{Ca}_3(\text{PO}_4)_2$ 15, Na_2HPO_4 10, KH_2PO_4 27.5, NaCl 10, KCl 5). On this diet the animals remained in fairly good condition even when completely phloridzinised for periods up to 3 weeks. The daily excretion of acetone bodies was about 4–8 mg. in normal rats, rising slightly on phloridzinisation to 5–15 mg.

The subcutaneous administration of adrenaline in doses of 0.5 mg. to phloridzinised rats caused a large rise in the excretion of acetone bodies. Figs. 1 and 2 show the effect in two animals: in (1) the total acetone bodies rose after administering adrenaline to 63 mg. and in (2) to 62 and 82 mg.; in each case the excretion fell to the normal level next day. In non-phloridzinised rats on the above diet or on a standard diet containing carbohydrates no rise in acetone body excretion was observed after the administration of adrenaline (see Fig. 1, days 1–5, and Fig. 3). In non-phloridzinised rats on a diet of fat with 5 % salts, adrenaline produced a small rise, the effect being more marked in young animals in which there was a greater ketosis. Thus Fig. 4 for an old rat shows a rise from 2.5 mg. acetone bodies to 5.7 mg. after adrenaline, and Fig. 5 for a young animal shows a larger initial ketosis of about 11 mg. gradually falling to 2 mg., with peaks of 23.9, 24.3, and 14.3 mg. after adrenaline. It was found that rats on this fat diet could not survive phloridzinisation for more than a day or two.

In view of the possibility that this rise in ketosis might be due to the acidosis produced by adrenaline, rats on the protein-fat diet + 5 % NaHCO_3 were phloridzinised and given adrenaline. The excretion of acetone bodies was at a higher level in these animals, as was expected from the finding of Wigglesworth [1924] that alkali produces ketosis, but adrenaline still caused a large rise comparable with that obtained on the protein-fat diet without alkali. Fig. 6 illustrates this, the rise being from about 20 mg. to 68 mg., falling to the original level next day.

Fig. 1. Abscissa: days. Ordinate: excretion in mg. per day of acetone bodies (acetone + acetoacetic acid + β -hydroxybutyric acid). Rat of about 165 g., put on protein-fat diet 4 days before beginning of experiment. Thickened portion of base line indicates period of phloridzinisation.

Fig. 2. Abscissa and ordinate as in Fig. 1. Rat of about 200 g., put on protein-fat diet one day before beginning of experiment; phloridzinised throughout experiment.

Fig. 3. Abscissa and ordinate as in Fig. 1. Rat of about 190 g., put on standard complete diet 9 days before beginning of experiment.

Fig. 4. Abscissa and ordinate as in Fig. 1. Rat of about 195 g., put on fat diet 2 days before beginning of experiment.

Fig. 5. Abscissa and ordinate as in Fig. 1. Young rat of about 130 g., put on fat diet 3 days before beginning of experiment.

Fig. 6. Abscissa and ordinate as in Fig. 1. Rat of about 195 g., put on protein-fat diet + 5% NaHCO_3 3 days before beginning of experiment. Thickened portion of base-line indicates period of phloridzinisation.

DISCUSSION.

In the experiments reported above we found a large rise in the excretion of acetone bodies with a quick return to the previous level. Since the effects of adrenaline only last for a few hours, this striking increase in the excretion of acetone bodies is no doubt even more intense than our figures for the

24 hour periods show. It seems that adrenaline may have a specific effect on fat metabolism, possibly in hastening β -oxidation and thereby the production of four-carbon residues. The increase in excretion of acetone bodies was only observed in cases where there was already a tendency to ketosis. If adrenaline exerts a specific stimulus to fat metabolism under normal conditions of diet, the amount of acetone bodies produced is not more than can be completely oxidised by the body.

That adrenaline causes a rise in metabolism is a well established fact; heat production and oxygen consumption are both increased. Lusk and Riche [1914], Palladin and Tichwinskaja [1925], and other observers have found no increase in protein metabolism. Increased combustion of carbohydrate might be suspected in view of adrenaline hyperglycaemia. But Boothby and Sandiford [1923] have pointed out that "the calorogenic action of adrenalin cannot be due simply to the presence of excess carbohydrate," for ingestion of sugar resulting in a blood-sugar much in excess of that in adrenaline hyperglycaemia gave only a small rise in heat production; an experiment on a diabetic showed no increase in metabolism after ingestion of 100 g. glucose but a marked increase after adrenaline with only a slight rise in blood-sugar. Stricker and Bouckaert [1924] injected glucose slowly into dogs till the blood-sugar was up to 0.3 % and found a slight fall in heat production, while adrenaline caused a sharp rise for a rather smaller hyperglycaemia. Thus the increase in metabolism after adrenaline cannot be wholly explained by the increase in free glucose.

The rise in the R.Q. after adrenaline found by most workers appears at first sight to rule out the possibility of an increase in fat metabolism. But a careful investigation of the figures given by Boothby and Sandiford [1923] and Krantz and Means [1926] shows that this rise is small and seems to precede the peak of heat production, pulse rate, blood pressure, etc. The apparent rise in R.Q. may be due to a washing out of CO_2 from the blood consequent on the decrease of alkaline reserve caused by adrenaline; thus Bornstein [1921] found that adrenaline caused increased lung ventilation and suggests that the temporary rise in R.Q. is due to loss of CO_2 and not necessarily to a larger combustion of carbohydrate. He observed that after the preliminary rise the R.Q. usually fell below normal.

The experiments of Hubbard and Wright [1921] on men and those of Raab [1926] on dogs may be quoted as more direct evidence that adrenaline affects fat and ketone body metabolism; these workers found an increase in acetone bodies in the blood after the administration of adrenaline, while Raab and also Fleisch [1926] observed a fall in blood-fat.

A temporary acidosis after adrenaline has been observed by Foa and Gatin-Gruzewska [1905], Peters and Geyelin [1917], Elias and Sammartino [1921], and others. In our experiments in which adrenaline was given to phloridzinised rats on a diet containing sufficient NaHCO_3 to produce an alkalosis, the rise in acetone bodies after adrenaline was comparable with

that obtained without the addition of bicarbonate to the diet. Also Wigglesworth [1924] found that an acidosis caused by NH_4Cl or $\text{NH}_4\text{H}_2\text{PO}_4$ reduced ketosis due to a fat diet or to alkali sometimes to a level below that found in rats under normal conditions. From these considerations it would seem that the acidosis is not responsible for the rise in excretion of acetone bodies after adrenaline.

In a recent paper Levine and Smith [1927] report that fasting for periods of from 4 to 7 days produced no appreciable ketosis in the rat, and that the output of acetone bodies during starvation was similar to that of animals consuming various diets. Thus in our experiments a lack of food intake during the adrenaline period cannot be responsible for the increased ketosis.

We suggest that the large rise in excretion of acetone bodies caused by adrenaline in phloridzinised or fat-fed rats may be due to a temporary intense stimulus to the oxidation of fat, the production of acetone bodies being increased beyond the capacity of the body to oxidise these substances.

SUMMARY.

1. The administration of adrenaline to phloridzinised rats on a protein-fat diet causes a very large temporary increase in the excretion of acetone bodies.
2. In non-phloridzinised rats on the protein-fat diet or on a standard diet containing carbohydrates, no rise in acetone body excretion is observed after the administration of adrenaline.
3. Adrenaline causes an increase in the ketosis of rats on a fat diet.
4. Addition of NaHCO_3 to the protein-fat diet does not prevent this effect of adrenaline.
5. It is suggested that the large rise in excretion of acetone bodies caused by adrenaline may be due to a temporary intense stimulus to the oxidation of fat.

We wish to thank Sir F. G. Hopkins and Mr J. B. S. Haldane for their interest in this work, which was done during the tenure by M. D. A. of a Fellowship at Newnham College and a part-time grant from the Medical Research Council.

REFERENCES.

- Boothby and Sandiford (1923). *Amer. J. Physiol.* **66**, 93.
Bornstein (1921). *Biochem. Z.* **114**, 157.
Elias and Sammartino (1921). *Biochem. Z.* **117**, 10.
Fleisch (1926). *Biochem. Z.* **177**, 461.
Foa and Gatin-Gruzeska (1905). *Compt. Rend. Soc. Biol.* **59**, 145.
Hubbard and Wright (1921). *J. Biol. Chem.* **49**, 385.
Krantz and Means (1926). *Proc. Soc. Exp. Biol. Med.* **23**, 698.
Levine and Smith (1927). *J. Biol. Chem.* **75**, 1.
Lusk and Riche (1914). *Arch. Int. Med.* **13**, 673.
Palladin and Tichwinskaja (1925). *Pflüger's Arch.* **210**, 436.
Peters and Geyelin (1917). *J. Biol. Chem.* **31**, 471.
Raab (1926). *Z. ges. exp. Med.* **49**, 179.
Stricker and Bouckaert (1924). *Compt. Rend. Soc. Biol.* **91**, 97.
Wigglesworth (1924). *Biochem. J.* **18**, 1203.

Note added December 3rd, 1927. While this paper was in the press, Burn and Ling (*J. Physiol.* **64**, xxii) published work reporting that adrenaline diminishes or inhibits ketonuria in rats on a diet of fat. The conditions were not the same as in our experiments: the doses of adrenaline were smaller and more frequent, and were given during the larger initial ketosis of the 2nd and 3rd days on the fat diet, not as in our experiments, after the animals had adapted themselves to the diet.