LXXXIX. FURTHER STUDIES ON THE LACTIC ACID, SUGAR AND INORGANIC PHOSPHORUS OF THE BLOOD OF RUMINANTS, (a) FOLLOWING ADRENALECTOMY AND (b) AFTER INTRA-VENOUS INJECTION OF INSULIN.

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IN a previous paper [Allcroft and Strand, 1933] we have shown that a marked increase occurs in the percentages of lactic acid and sugar in the blood of sheep after muscular exercise and/or excitement, and it seemed important to determine whether these changes would also occur after adrenalectomy. While there are numerous references to the effect of adrenalectomy in dogs and small laboratory animals, we can find only one reference to similar work in the ruminant. Moore and Purinton [1901], working on two goats and two kids, found that the operation was fatal in three out of the four cases.

Briggs *et al.* [1923-24] state that after insulin injections in normal animals, there is a decrease in glucose, phosphorus and potassium in the blood with an increase in blood-lactic acid. Best and Ridout [1925] state that the lactic acid content of the blood of dogs does not significantly increase during insulin hypoglycaemia. Cori [1925] reports that insulin hypoglycaemia was attended by no definite changes in the lactic acid of either fasting rabbits or cats: later, however [1931; 1932], this worker reports an increase in lactic acid and a decrease in phosphorus after injection of insulin in normal rabbits, but after adrenalectomy these changes were not observed. Katayama and Killian [1926-27] also conclude that insulin produces an increase in the lactic acid of the blood of rabbits and human beings and note that in the rabbit the changes in concentration of lactic acid are very slight in comparison with the drop in blood-sugar. These rather contradictory reports led us to attempt to study the changes in lactic acid, sugar and inorganic phosphorus of the blood of sheep after injection of insulin. Though it was not possible to complete the study it is thought advisable to report the observations which were made.

Technique.

The sheep were brought in from grazing and put in metabolic cages for 18-24 hours before operation. Food was withheld during this period but water was available. The animals were anaesthetised by ether and the wool was cut short, but not shaved, over the skin of the abdomen between the costal margin and the umbilicus. An oblique incision (about 8 in. long) was then made in the loin stretching from the lower border of the 12th rib on the right side

towards the umbilicus. By retraction of the viscera, the adrenal gland was readily exposed and the small vein crossing in front of it from the parietes to the inferior vena cava was doubly ligated. Narrow tapes were then placed with care under the vena cava above and below the site of the gland. By gently pulling on these it was possible to control the bleeding during the removal of the gland, which was the next step. After removal, any obvious small veins or arteries were tied and the longitudinal slit which is unavoidably made in the vena cava, as a result of the excision of the gland (on account of the short length of the adrenal vein) stitched by fine linen thread on fine needles, and a small piece of wet cotton wool placed over the suture line to prevent leakage. The abdominal wound was then closed and the animal allowed to recover from this first operation. The removal of the left adrenal gland followed in 8–20 days. In this case the length of the adrenal vein was sufficient to permit of the removal of the gland without damaging the vena cava. The animals recovered well from both operations, without any suppuration of the wounds.

In a few cases cortico-adrenal extract was injected after bilateral adrenalectomy to maintain the animal in good condition. The extract was made by Grollman and Firor's method [1933] and assayed by intraperitoneal injection into month-old adrenalectomised rats. If the extract were potent the rats gained 1–4 g. in weight daily and appeared to be in a normal condition. Occasional batches of extract were not potent, and after injection of these the rats lost weight daily showing typical adrenal insufficiency symptoms such as loss of appetite, muscular weakness, general apathy and often convulsions just before death, which resulted in 2–4 days after the operation.

Although the extracts were as a rule potent as judged by their effect on growing rats, it is not certain, owing to the limited number of experiments, whether their injection into adrenalectomised sheep really prolonged life (see Table I). We gained the impression however that the injected animals were in better condition than the controls.

Owing to the short survival period of bilaterally adrenalectomised sheep it was found necessary to carry out the excitement experiments as soon as possible and the second day after the final operation was chosen since by that time the blood-sugar had assumed a low level. Blood samples were taken from most of the animals before unilateral adrenalectomy, in the period between unilateral and complete adrenalectomy, and after the final operation. The technique and times of sampling were essentially the same as in the earlier work except that sampling was continued for only 2 hours following the excitement, since this period covered the major changes which we had noted.

In the insulin injection experiments, insulin was injected into the left jugular vein and samples of blood were taken from the right jugular vein, before injection and at intervals of 15, 30, 45, 60, 90, 120, 180, 240 and 300 minutes afterwards. The amount of insulin injected per kg. body weight is given in each graph. In the case of the one adrenalectomised sheep to which insulin was administered it was not possible to obtain the full series of samples owing to convulsions, which occurred 50 minutes after the insulin injection.

Lactic acid was estimated by the method of Friedemann *et al.* [1927], blood-sugar by the Hagedorn and Jensen [1920] method and inorganic phosphorus by Briggs method [1922]. For all estimations the blood-proteins were precipitated within 1–2 minutes after the blood sample was drawn.

The results of parts (a) and (b) are given and discussed separately.

(a) The following table (Table I) gives the results of adrenalectomy on the blood constituents.

Table I.

Results expressed in mg./100 ml. whole blood.

Sheep No.	Before unilateral adrenalectomy			After unilateral adrenalectomy			After complete adrenalectomy			Survival period in (hours)	Remarks
	Lactic acid	Blood-sugar	Inorg.-P	Lactic acid	Blood-sugar	Inorg.-P	Lactic acid	Blood-sugar	Inorg.-P		
167	15.23	61.80	—	10.40	57.40	—	5.80	45.96	8.44	52	Left gland removed 8 days after right. In good condition first day after complete adrenalectomy. Insufficiency symptoms set in about 9 a.m. second day. No C-A extract injected
	15.57	60.04	—	10.12	61.80	7.76	7.00	40.68	8.64		
	—	—	—	7.72	61.80	7.76	6.05	35.40	9.64		
	—	—	—	—	—	—	8.95	40.68	9.64		
125	—	—	—	22.05	79.40	—	8.45	35.40	—	58	Left gland removed 10 days after first operation. In good condition first day after complete adrenalectomy. On second day insufficiency symptoms were marked. No C-A extract injected
	—	—	—	19.50	81.16	—	6.26	35.40	—		
	—	—	—	20.20	—	—	8.45	37.16	10.72		
	—	—	—	—	—	—	8.45	34.52	11.64		
1	17.45	55.64	3.94	—	—	—	—	—	—	—	Left gland removed 7 days after first operation. Died a few hours after second operation due to haemorrhage from inferior vena cava
	15.50	52.12	4.21	—	—	—	—	—	—		
2	13.30	52.00	4.47	—	—	—	—	—	—	34	Left gland removed 8 days after right. In good condition day after complete adrenalectomy. Two blood samples taken 9.30 and 10 a.m. (Nos. 1 and 2). Then 10 ml. of C-A extract injected intraperitoneally. Two more samples (3 and 4) taken 15 and 30 minutes after injection. No insufficiency symptoms apparent but sheep died a few hours after injection of toxic extract No. 5*
	47.70	68.84	6.08	—	—	—	(1) 11.10	41.56	10.64		
	17.70	73.24	5.42	—	—	—	(2) 13.75	44.20	9.86		
	27.90	74.12	5.42	—	—	—	(3) 13.75	65.32	10.80		
3	—	—	—	—	—	—	(4) 14.50	66.20	11.16	60	Left gland removed 18 days after first operation. In good condition next day and following day during which it received 30 ml. extract in 5 ml. injections. Was in good condition 6.30 p.m. on second day but collapsed suddenly and died 11 p.m. same night
	15.00	71.48	6.03	12.30	64.44	4.42	7.60	47.72	7.30		
	13.05	74.12	5.32	19.00	60.04	5.09	6.40	38.92	8.14		
	14.25	73.24	5.44	20.50	66.20	5.17	7.25	43.32	7.89		
4	18.30	61.80	5.53	11.80	62.68	2.79	—	—	—	—	Left gland removed 16 days after first operation. Died the morning following the second operation due to tying of the vena cava
	11.10	62.68	4.98	9.70	65.32	2.79	—	—	—		
5	12.56	62.68	4.66	10.40	68.84	2.56	—	—	—	52	Left gland removed 6 days after right. 5 ml. C-A extract (No. 11) injected at hourly intervals during day following complete adrenalectomy. Animal in very good condition. Samples 1 and 2 taken day after second operation and samples 3 and 4 the next morning. After No. 4 sample was taken insulin (0.75 units/kg. body weight) was injected. 50 minutes afterwards animal had convulsions. C-A extract and large quantities of glucose solution were injected but sheep did not recover and died 7 hours after insulin injection
	24.00	70.60	5.65	—	—	—	(1) 11.40	54.76	7.94		
	26.70	69.72	5.60	—	—	—	(2) 11.60	54.76	7.68		
	26.00	70.60	5.60	—	—	—	(3) 12.15	62.68	12.24		
	—	—	—	—	—	—	(4) 10.40	55.64	13.00		

* This extract was found to be highly toxic by injecting 0.5 ml. of it intraperitoneally into mice.

The effects of excitement are shown in the curves of Figs. 1-4.

Sheep Nos. 167 and 125 (Figs. 1 and 2) were subjected to a barking dog but showed singular lack of interest in the proceedings. After the 120 minute sample had been taken one of the sheep (No. 125) was exercised for 5 minutes and then samples taken immediately and 15 minutes after. The dog barking caused no significant alteration in the level of any of the three constituents studied in

Fig. 1.

Fig. 2.

Fig. 1. Effect of excitement (dog barking) on sheep No. 167 bilaterally adrenalectomised. Ordinate: mg. per 100 ml. whole blood.

Fig. 2. Effect of excitement (dog barking) on sheep No. 125 bilaterally adrenalectomised. 10 minutes' exercise between 120 and 130 minutes. Ordinate: mg. per 100 ml. whole blood.

Fig. 3.

Fig. 4.

Fig. 3. Effect of excitement (by explosion of cracker) on sheep No. 3 bilaterally adrenalectomised. Ordinate: mg. per 100 ml. whole blood.

Fig. 4. Effect of excitement (by explosion of cracker) on sheep No. 4 unilaterally adrenalectomised. Ordinate: mg. per 100 ml. whole blood.

either animal, and the changes in lactic acid and sugar which occurred in sheep No. 125 after exercise are so small as to be of doubtful significance (Fig. 2).

Sheep Nos. 3 and 4, the former bilaterally, and the latter unilaterally adrenalectomised, were subjected to the explosion of a medium-sized firework placed about 4 ft. from them. Sheep No. 3 (Fig. 3) showed no alteration in lactic acid or inorganic P but the curve for sugar showed a lower level after the explosion, returning to the pre-experimental level within 30 minutes. In sheep

No. 4 the only change was a slight elevation of the blood-sugar immediately after the explosion (Fig. 4). This increase in blood-sugar and the normal low level of the inorganic P is, in our opinion, due to the fact that the animal is not completely adrenalectomised.

DISCUSSION.

(a) It can be seen from the above results that the blood-lactic acid was lower after complete adrenalectomy than before or after unilateral adrenalectomy. This is contrary to the conclusions of Britton and Silvette [1932] with cats, but in agreement with those of Hastings and Compere [1930-31] with dogs. The blood-sugar level was also considerably lowered after complete adrenalectomy. Injection of C-A extract definitely raised the blood-sugar in one sheep, No. 2, and possibly diminished the fall in another, No. 5 [cf. Britton and Silvette, 1932; Wyman and Walker, 1929].

The inorganic P was considerably raised after complete adrenalectomy. It was observed too in every case after the second gland was removed that there was a marked increase in the blood concentration so that it was often difficult to get a series of blood samples. Just before death the usual symptoms of adrenal insufficiency were present, *viz.* refusal of food, muscular weakness, prostration, decrease in body temperature and finally convulsions. These observations, increase in inorganic P, anhydraemia and insufficiency symptoms, agree with those made by Britton and Silvette [1932], Britton [1932] and Lucas [1926].

Banting and Gairns [1926] state that the lowering of the blood-sugar does not depend on the absence of the adrenals but is the result of the shock of the operation. If this were so one would expect a gradual rise in the blood-sugar as the animal recovered from the operation, but in no instance was this observed. None of the animals survived for a longer period than 60 hours after complete adrenalectomy. In the cases where cortico-adrenal extract was injected (except in sheep No. 5) the short survival period was probably due to the fact that the extract was not sufficiently potent to prolong the life of the animal. On the same extract however we were able to keep completely adrenalectomised month-old and adult rats indefinitely. It is extremely probable that sheep No. 5 died of insulin shock since it has been shown that adrenalectomy renders other animals hypersensitive to insulin [Ellsworth and Weinstein, 1933; Tada, 1931; Lewis and Magenta, 1925; Houssay and Lewis, 1923]. Moore and Purinton [1901] record survival periods for two bilaterally adrenalectomised kids of 49 and 60 hours, while one goat survived 8 days and in another the results were not fatal.

Effect of excitement. It is evident that in completely adrenalectomised sheep no significant alteration occurs in the levels of lactic acid, sugar or inorganic P of the blood after the same type of emotional stimuli which were found to cause marked rises in normal sheep [Allcroft and Strand, 1933]. This confirms the views of Lumley and Nice [1930], who showed that after excitation induced by shocks sufficient to cause struggling in adrenalectomised rats, no increase in the blood-sugar occurred, while in normal rats under similar conditions increases up to 86 % were observed. Britton [1933] also reports that none of the measures which normally produce hyperglycaemia in cats, *e.g.* adrenaline, anaesthesia or excitement, has any significant influence on the blood-sugar level of adrenalectomised animals.

(b) Figs. 5-8 show graphically the changes which occurred after insulin injection in the four unoperated sheep. Figs. 5 and 7 show results obtained on the same sheep with an interval of 16 days between the experiments.

In Table II are given the results of the few blood samples which were secured from the completely adrenalectomised sheep, and also in chronological sequence the therapeutic treatment administered during the convulsive stages. Though

Fig. 5.

Fig. 6.

Fig. 5. Effect of intravenous injection of insulin (2 units per kg.) on normal sheep No. 9. 25. ix. 33. Ordinate: mg. per 100 ml. whole blood.

Fig. 6. Effect of intravenous injection of insulin (2 units per kg.) on normal sheep No. 13. 25. ix. 33. Ordinate: mg. per 100 ml. whole blood.

Fig. 7.

Fig. 8.

Fig. 7. Effect of intravenous injection of insulin (2 units per kg.) on normal sheep No. 9. 11. x. 33. Ordinate: mg. per 100 ml. whole blood.

Fig. 8. Effect of intravenous injection of insulin (3 units per kg.) on normal sheep No. 10. 11. x. 33. Ordinate: mg. per 100 ml. whole blood.

the animal had been maintained in good condition with cortico-adrenal extract up to the commencement of the experiment the subsequent injections of glucose and cortico-adrenal extract were unable to prevent death 7 hours after injection of insulin.

It will be seen that in only one case (Fig. 5, sheep No. 9) did the blood-lactic acid show any definite increase after intravenous injection of insulin. The samples obtained at 45 and 300 minutes after injection of insulin into the adrenalectomised sheep (see Table II) gave high values for lactic acid associated

Table II. *Effect of insulin injection on a completely adrenalectomised sheep.*

27.2 kg. weight (0.75 unit insulin per kg.) 20.4 units injected.
Results expressed in mg./100 ml. whole blood.

	Lactic acid	Blood-sugar	Inorganic P	Remarks
Before injection	12.15	62.68	12.24	—
	10.40	55.64	13.00	Injection of 0.75 unit insulin per kg. body weight
15 mins. after	11.60	45.96	12.16	—
30 "	10.05	40.68	11.54	—
45 "	23.75	36.28	12.50	—
50 "	—	—	—	Convulsions began. 11 ml. glucose intravenously
70 "	—	—	—	11 ml. glucose intravenously
83 "	—	—	—	11 ml. glucose intravenously
95 "	—	—	—	11 ml. glucose intravenously + 0.5 ml. adrenaline subcutaneously
210 "	—	—	—	60 ml. glucose subcutaneously
300 "	20.10	57.40	10.98	—

with the hypoglycaemic convulsions. The changes in the blood-sugar correspond to those reported in the literature, although the degree of hypoglycaemia produced was not the same in all cases, and the low level seemed more prolonged in the ruminant than in simple-stomached animals. The fall in inorganic P agrees with the work of Cori and Cori [1932] on rabbits and of Eadie *et al.* [1925] on dogs and rabbits. The former observed that the level was lowest 45 minutes after injection and gradually returned to the original level. Eadie *et al.* [1925] state that the blood-phosphate fall is simultaneous with that of blood-sugar, but the rise to normal occurs before that of sugar and in some cases may continue above the normal level. In our own work the lowest levels lie between 45 and 60 minutes after injection and in two cases (Figs. 7 and 8) there was a rise to above the normal level. It is possible that this return to a higher level was delayed in Figs. 5 and 6 and might have occurred after the last samples were taken. Ellsworth and Weinstein [1933] observed similar phenomena in dogs whether normal, or completely adrenalectomised and maintained on cortical extract: one adrenalectomised sheep however showed only a very slight fall in inorganic P.

It is obvious that adrenalectomy increases the sensitivity of the sheep to insulin just as it does that of other animals. At the same time it is evident that the normal ruminant can tolerate much larger doses of insulin without occurrence of convulsions than can simple-stomached animals. Peterson *et al.* [1931] report that they lowered the blood-sugar in cows to values as low as 17 mg. per 100 ml. by insulin injections and yet failed to induce convulsions.

SUMMARY.

1. A description is given of the surgical technique used for adrenalectomy operations on sheep.
2. After bilateral adrenalectomy in sheep the blood-sugar and lactic acid levels were definitely lowered while that of inorganic P was raised.
3. No change occurred in blood-sugar and lactic acid of adrenalectomised sheep when they were subjected to such types of excitement and muscular exercise as were known to raise them in normal sheep.
4. There was no definite change in the blood-lactic acid of normal sheep after insulin injections.

5. The hypoglycaemia produced by insulin injection in normal ruminants was similar to that in other animals except that no sign of convulsions was observed even after large doses of insulin.

6. The changes in blood-inorganic P after insulin corresponded to those found in other animals.

7. Bilateral adrenalectomy increases the sensitivity of the ruminant to insulin.

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