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OVINE KETOSIS. IV. THE EFFECT OF ALLOXAN ON THE KETONE BODY AND BLOOD SUGAR LEVELS OF MERINO WETHERS*

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INTRODUCTION

It has been previously shown that the administration of alloxan leads to the destruction of the islets of Langerhans in the pancreas (Dunn, Sheehan & McLetchie, 1943) with a subsequent development of a true diabetic condition which results in a simultaneous increase of both blood sugar and ketone body levels.

A small number of workers have previously studied this condition in ruminants. Jarrett (1946) administered alloxan intravenously to sheep in doses varying from 90 to 200 mg per Kg body weight. Only blood sugar values were determined and these rose to levels of 230, 250 and 310 mg per cent in three of the animals which survived for a long period (14, 47 and 53 days respectively). McCandless, Woodward & Dye (1948) also administered alloxan to sheep in doses varying from 75 to 125 mg per Kg. The blood sugar and ketone body values, obtained sporadically during a ten day period, ranged from 146 to 218 mg per cent for the former and 11 to 51 mg per cent for the latter. Survival times varied, ranging from only 85 hours to 157 days. Schultz & Smith (1951) dosed one goat with 90 mg of alloxan per Kg. As a result the ketone body and blood sugar levels rose to 60 and 200 mg per cent respectively and the animal survived for 14 days. Finally Goetsch (1957) administered 100 mg of alloxan per Kg to non-pregnant ewes. In this instance blood sugar and ketone body levels were determined only at 24 and 48 hours after the dosing of the drug. During this period the former were found to rise on an average to a maximum of 260 mg per cent and the latter to one of 4·9 mg per cent.

In the present experiment the animals were dosed with approximately 50 mg of alloxan per Kg and both blood sugar and ketone body levels determined. In addition the latter were partitioned into their individual fractions.

EXPERIMENTAL

Animals

Five good-conditioned, adult, Merino wethers were used. The animals were housed in individual cages.

Diets

The animals received a daily diet consisting of 2,000 gm fresh green lucerne and 350 gm concentrates (oats 60%, wheat bran 20%, crushed maize 13%, peanut meal 5%, salt 1% and lime 1%).

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Sampling

Samples of jugular blood (5 ml) were obtained each morning at 8 a.m. before feeding.

Dosing

The animals received the following doses of alloxan:—

Sheep No.	*Alloxan in mg per Kg body weight*
1412	51
3490	47
3552	48
2465	51
2932	51

The drug was dissolved, immediately before use, in 200 ml of normal saline solution and injected into the jugular vein. Protamine Zinc Insulin, in doses of 1·8 u per Kg, was administered subcutaneously to two of the sheep (No. 2465 and 2932) at 5, 6, 7 and 14, 15 and 16 days after the injection of alloxan.

Analytical

In all samples the concentrations of the individual ketone bodies (acetone, acetoacetic and β-hydroxybutyric acids) were estimated separately (Procos, 1961). Blood sugar concentrations were determined by Lehmann & Silk's (1952) modification of the Folin & Wu (1920) method.

Results

Time of survival

The survival times, in days, of the five sheep are listed below:—

Sheep No.	*Days of survival*
3552	92
1412	11
3490	14
2465	25
2932	25

Sheep No. 3552 remained normal throughout the experimental period (43 days) and for a further 49 days when the animal was slaughtered. Of the others, sheep No.1412 , 3490 and 2932 became comatose on the 11th, 14th and 25th day respectively after the administration of the alloxan and were slaughtered immediately. Sheep No. 2465 appeared dull and was found to have developed a diarrhoea on the 24th day. This animal was killed *in extremis* on the following day. At this stage it must be emphasised that in all probability both sheep No. 2465 and 2932 would have succumbed earlier, had not insulin therapy been instituted.

Food consumption

Apart from sheep No. 3552, the appetite of which remained unaltered throughout, all the other animals displayed decreased appetites. The average daily food consumption of these animals was as follows:—

Sheep No.	*Green lucerne in gm*	*Concentrates in gm*
1412	620	Very little
3490	670	nil
2465	1,780	248
2932	1,400	256

All the above animals refused to eat their food two to three days before becoming comatose.

Blood sugar levels

Following the administration of alloxan, the blood sugar levels of all the experimental animals increased abruptly and remained consistently high thereafter except when, as in the case of sheep No. 2465 and 2932, insulin treatment was instituted (Tables 1 and 2).

In order to avoid confusion these values were omitted from the calculations of the average blood sugar values presented below:—

Sheep No.	*Average blood sugar level in mg per cent*
3552	181
1412	149
3490	178
2465	165
2932	173

It is apparent that, despite the differences in survival times, a close similarity existed between the blood sugar levels of all the sheep.

Total ketone body levels

The total ketone body levels of the experimental animals increased significantly following the administration of alloxan (Tables 1 and 2). However, in contrast to the blood sugar levels, they did not reach an equilibrium but continued to rise steadily with the exception of sheep No. 3552 whose levels increased somewhat at first, but remained constant thereafter (average of 19·2 mg per cent).

The individual ketone bodies

As the total ketone bodies increased, it became apparent that there was a tendency for the acetone fraction to increase and the β-hydroxybutyric acid fraction to decrease. Before the dosing of the drug the former fraction represented on an average 4 per cent of the total ketone bodies (range 0 to 17 per cent) while after dosing it increased to 47 per cent (range 45 to 56 per cent). On the other hand, the β-hydroxybutyric acid fraction was on an average 78 per cent of the total (range 48 to 100 per cent) at pre-dosing levels and decreased to 20 per cent (11 to 27 per cent) after dosing.

Again sheep No. 3552 was different in that its acetone percentage remained at 13 per cent (range 3 to 20 per cent) throughout the entire post-dosing period.

The effect of insulin

Insulin administration resulted as expected in a decrease in blood sugar and ketone body levels. However, both the degree of response and the time at which it occurred were somewhat different in the two animals treated. It was further found that under the influence of insulin the acetone fraction tended to diminish and the β-hydroxybutyric acid fraction to increase.

TABLE 1.—*The effect of alloxan administration on the blood ketone body and sugar levels (mg per cent) of adequately fed Merino wethers*

Days after alloxan administration	Sheep No. 1412					Sheep No. 3490					Sheep No. 355?				
	A	AA	BOH	TKB	BS	A	AA	BOH	TKB	BS	A	AA	BOH	TKB	S
0	0·00	1·36	1·27	2·63	56·0	0·00	1·00	1·15	2·15	51·0	0·50	1·18	2·70	4·38	49·0
1	—	—	—	—	—	1·60	1·68	2·37	5·65	196·0	—	—	—	—	—
2	6·00	6·70	9·02	21·72	136·5	—	—	—	—	—	0·00	0·00	0·00	0·00	191·0
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	9·40	6·17	9·65	25·22	181·0	—	—	—	—	—
5	16·60	14·13	6·93	37·66	153·0	—	—	—	—	—	0·50	1·04	1·51	3·05	191·0
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	15·70	13·55	5·68	34·93	150·0	16·00	13·11	7·90	37·01	174·0	0·60	1·31	4·65	6·56	208·0
9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	37·00	21·64	7·34	65·98	156·0	23·40	18·35	10·37	52·12	156·0	0·00	0·00	1·86	1·86	67·0
11	—	—	—	—	—	35·30	24·53	18·97	78·80	201·0	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	0·90	7·98	17·67	27·45	115·0
19	—	—	—	—	—	—	—	—	—	—	1·10	1·36	1·33	3·79	215·0
21	—	—	—	—	—	—	—	—	—	—	1·75	1·25	12·41	15·41	208·0
22	—	—	—	—	—	—	—	—	—	—	1·20	0·44	6·26	7·90	205·0
25	—	—	—	—	—	—	—	—	—	—	2·00	3·19	7·98	13·17	201·0
27	—	—	—	—	—	—	—	—	—	—	2·40	5·11	12·63	20·14	180·0
28	—	—	—	—	—	—	—	—	—	—	3·00	4·24	11·20	18·44	186·0
32	—	—	—	—	—	—	—	—	—	—	2·70	2·98	17·14	22·82	186·0
33	—	—	—	—	—	—	—	—	—	—	2·40	1·97	17·16	21·53	194·0
40	—	—	—	—	—	—	—	—	—	—	4·15	5·08	11·72	20·95	155·0
41	—	—	—	—	—	—	—	—	—	—	1·60	2·43	30·14	34·17	155·0
43	—	—	—	—	—	—	—	—	—	—	2·20	3·80	18·93	24·93	172·0

A = acetone; AA = acetoacetic acid; BOH = β-hydroxybutyric acid; TKB = total ketone bodies; BS = blood sugar

TABLE 2.—*The effect of alloxan and insulin administration on the blood ketone body and sugar levels (mg per cent) of adequately fed Merino wethers*

Days after alloxan administration	Protamine zinc insulin units administered	Sheep No. 2465					Sheep No. 2932				
		A	AA	BOH	TKB	BS	A	AA	BOH	TKB	BS
0	—	0·10	0·17	3·90	4·17	49·0	0·00	0·00	2·65	2·65	56·0
0	—	0·10	0·17	2·98	3·25	49·0	0·00	0·00	2·87	2·87	54·0
0	—	0·00	0·00	2·73	2·73	49·0	0·00	0·00	0·00	0·00	56·0
0	—	0·20	1·44	3·16	4·80	49·0	0·60	2·70	0·19	3·49	54·0
1 (9 a.m.)	—	0·50	0·00	6·00	6·50	34·0	0·00	0·00	6·77	6·77	44·0
1 (3 p.m.)	—	—	—	—	—	191·0	—	—	—	—	208·0
2	—	3·40	6·24	0·63	10·27	160·0	1·75	7·89	11·22	20·86	191·0
3	—	9·60	13·14	6·27	29·01	152·0	11·05	7·41	33·21	51·67	177·0
4	—	18·10	15·09	18·18	51·37	194·0	18·10	18·78	15·00	51·88	208·0
5	1·8 u/Kg body weight	—	—	—	—	169·0	—	—	—	—	170·0
6	1·8 u/Kg body weight	—	—	—	—	—	—	—	—	—	—
7	1·8 u/Kg body weight	2·40	1·89	20·88	25·17	126·0	1·65	5·73	0·00	7·38	44·0
8	—	0·20	1·23	9·30	10·73	40·0	0·35	1·08	9·90	11·33	42·0
9	—	0·40	0·00	9·54	9·94	174·0	0·00	0·00	10·14	10·14	167·0
10	—	5·40	3·60	21·39	30·39	112·0	8·80	11·49	2·70	22·99	133·0
11	—	7·10	4·77	28·65	40·52	140·0	12·35	12·84	3·54	28·73	167·0
12	—	—	—	—	—	129·0	—	—	—	—	148·0
14	1·8 u/Kg body weight	13·40	10·62	39·84	63·86	174·0	16·60	7·80	32·88	57·28	174·0
15	1·8 u/Kg body weight	6·75	11·91	14·19	32·85	112·0	2·60	11·31	1·32	15·23	68·0
16	1·8 u/Kg body weight	2·40	7·44	0·26	10·10	122·0	1·95	7·89	3·44	13·28	89·0
17	—	2·60	8·05	5·34	15·99	133·0	2·60	8·05	8·60	19·25	128·0
18	—	4·00	8·78	24·47	37·25	129·0	4·65	7·44	21·93	34·02	131·0
21	—	10·00	22·59	10·82	43·41	151·0	11·20	7·65	33·67	52·52	160·0
22	—	20·20	11·76	34·27	66·23	179·0	14·20	12·03	7·25	33·48	147·0
23	—	40·00	32·12	7·44	79·56	182·0	22·80	25·35	24·58	72·73	190·0
24	—	49·40	20·47	38·80	108·67	193·0	30·15	20·25	12·14	62·54	190·0
25	—	55·70	33·02	33·03	121·75	203·0	35·30	19·61	17·16	72·07	201·0

A = acetone; AA = acetoacetic acid; BOH = β-hydroxybutyric acid; TKB = total ketone bodies; BS = blood sugar

Discussion

It is generally accepted that alloxan destroys the β-cells of the islets of Langerhans in the pancreas and that this destruction results in an insulin deficiency. In turn insulin deficiency leads to an increase in blood sugar levels due to three possibilities:—

(*a*) through the inhibition of the hexokinase reaction by which glucose is phosphorylated to glucose-6-phosphate and is thereby allowed to enter the general metabolic scheme (Price, Cori & Colowick, 1945);

(*b*) through the reduction of the permeability of the cell to glucose (Levine, Goldstein, Klein & Huddlestun, 1949); and

(*c*) through the depression of synthetic reactions by lowering the energy potential of the cell (Beloff-Chain & Pocchiari, 1960).

Whatever the mechanism, the derangement of the carbohydrate metabolism leads to an enhanced catabolism of fat for the production of energy (Cumming & Morrison, 1960). In turn, fat catabolism results in the production of acetyl CoA which, in view of the impairment in carbohydrate metabolism cannot be metabolised fast enough via the Krebs cycle and therefore accumulates. This accumulation leads to the eventual condensation of two molecules of acetyl CoA to form acetoacetyl CoA and hence acetoacetic acid, β-hydroxybutyric acid and acetone.

Two interesting facts have emerged from this study. The one concerns the increase of the acetone fraction at the expense of the β-hydroxybutyric one. It is known that aceto-acetic acid, the parent ketone body substance, can be either spontaneously decarboxylated to acetone or it can be reduced to β-hydroxybutyric acid via β-hydroxybutyric dehydrogenase with reduced diphosphopyridine nucleotide (DPNH) as the hydrogen donor. A similar increase in acetone was previously shown to occur during starvation (Procos, 1962) and it was postulated that, in view of the findings of Kulka, Krebs & Eggleston (1961), this was probably due to a decreased supply of DPNH. DPNH is produced during both, the catabolism of glucose via the Embden-Meyerhof (E-M) pathway and the normal function of the Krebs cycle. Since the fact that the ketone body levels increased is itself an indication of Krebs cycle malfunction while the increase in blood sugar points to a non-utilisation of this metabolite, it is not surprising that a shortage of DPNH occurred. The fact that insulin increased the proportion of β-hydroxybutyric acid therefore indicates, that one of its sites of action is probably the E-M glycolytic pathway. This is in agreement with the *in vitro* findings of Siperstein & Fagan (1958).

The other interesting point is the abnormal behaviour of sheep No. 3552. This particular animal, although dosed with the same amount of alloxan, survived for a much longer period, its appetite remained unaltered, its total ketone body level reached an equilibrium at 19 mg per cent and in addition its acetone fraction was low compared with that of the other animals. In contrast its blood sugar levels remained high throughout the experimental period (average 181 mg per cent).

It has been maintained that alloxan administration differs from pancreatectomy in that it often leads to a "meta-diabetic" state presumably due to the retention of some insulin-producing capacity (Soskin & Levine, 1952). This theory, however, is not supported by the present findings since the presence of even small amounts of insulin would have resulted in a lowering of the blood sugar levels which was not the case. At present, the only other possible explanation that can be offered, which would reconcile all the above findings, is that part of the animal's energy requirements was provided by the food, the other part coming from fat catabolism.

The fermentation of the food by the rumen organisms results in the production of acetic, propionic and small amounts of butyric acid. Of these, acetic and propionic are metabolised via the Krebs cycle with the formation of DPNH. In addition propionic acid gives rise to oxaloacetate (Flavin, Ortiz & Ochoa, 1956).

The presence of the latter would lead to the utilisation of some of the acetyl CoA produced during the catabolism of fat, thus enabling the organism to maintain an equilibrium in ketone body production, while the former would participate in the reduction of the major portion of the acetoacetic acid produced, to β-hydroxybutyric acid.

Summary

Administration of alloxan led to an increase of both the blood sugar and ketone body levels of the wethers. Of the individual ketone bodies, acetone increased to a far greater extent than acetoacetic or β-hydroxybutyric acid. Insulin was shown to induce temporary relief by decreasing the ketone body and blood sugar levels.

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