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OVINE KETOSIS. III.— THE EFFECT OF STARVATION ON THE BLOOD SUGAR AND KETONE BODY LEVELS OF WETHERS

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INTRODUCTION

The ketoses of preparturient ewes and lactating cows have long been associated with a reduced food intake coupled with a failure of the compensatory gluconeogenic mechanism controlled by the adrenal cortex (Dyer, Roberts, Blampied & Fincher, 1953). In 1941, Groenewald, Graf, Bekker, Malan & Clark reported the production of typical pregnancy disease in heavily pregnant ewes by a sudden reduction in food intake, but subsequent attempts to repeat these results both at Onderstepoort and elsewhere, have not been very successful. In order to clarify the role played by starvation *per se*, it was decided to assess its effect on non-pregnant sheep.

A review of the literature showed that only few workers have attempted to study the effect of starvation on the ketone body and blood sugar levels of non-pregnant sheep. Allcroft & Strand (1933) starved two sheep for periods of seven and six days respectively. The first period of starvation resulted in a blood sugar fall from 57 to 45 mg per cent, while the second resulted in a fall from 68 to 42 mg per cent. Sampson & Boley (1940) fasted five non-pregnant ewes for eight days and observed a fall in blood sugar from 53 to 36 mg per cent and a rise in ketone bodies from 1·8 to 4·7 mg per cent. Robertson & Thin (1953) starved a non-pregnant ewe with subclinical ketosis for four days and as a result the total ketone body level of the animal rose from 11·7 to 22·3 mg per cent. In view of the fact that food is retained in the rumen for as long as 48 hours, this period of starvation is too short in the author's opinion. White, Christian & Williams (1956) subjected twelve wethers to a nine day period of starvation preceded by a long period (84 days) of gradual food reduction. During the fast the blood sugar levels fell on an average from 38 to 31 mg per cent while the ketone body levels rose from 1·7 to 9·0 mg per cent. In this case, the animals were obviously conditioned to a state of semi-starvation, so that the stress that could be expected to accompany abrupt food deprivation was greatly minimised.

In the present experiment wethers were used in preference to ewes in order to eliminate the effect of sex hormones since it has been previously shown that oestrus has an influence on the ketone body levels of ewes (Procos, 1961) and also that sex plays an important role on the degree of reaction of animals to starvation (Keys, 1950). In addition the ketone bodies were partitioned into their individual fractions (acetone, acetoacetic and β-hydroxybutyric acids). This was thought to be of particular importance in view of the fact that the concentration of acetone

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in the blood of ketotic sheep appears to rise at the expense of acetoacetic and β-hydroxybutyric acids (Robertson & Thin, 1953; Procos, 1959). Two diets were used during the pre-fasting period, one consisting of lucerne hay and cow meal and the other one of lush green lucerne and cow meal. These two diets were chosen because it was previously noticed that the blood sugar levels of sheep fed these two diets seemed to be consistently different from each other.

Experimental

The experiment lasted 72 days in all (20 August to 29 October, 1958). The pre-fasting period was 33 days, starvation lasted eight days and the post-fasting period was 31 days.

Animals

Six good conditioned, adult Merino wethers, weighing an average of 88 lb were used. The animals were kept individually in small, covered pens and weighed at approximately seven day intervals.

Diets

Three wethers (No. 1, 2, 3) received a daily ration consisting initially of 2,000 gm and a week later of 3,000 gm lush green lucerne, 200 gm cow meal and 10 gm salt/bone meal (2: 1) mixture. The remaining animals (No. 4, 5, 6) received 500 gm lucerne hay, 200 gm cow meal and 10 gm salt/bone meal mixture.

The dry weight of the green lucerne was found to be one sixth of its fresh weight and it is, therefore, obvious that the quantities of lucerne hay and green lucerne above, were equivalent on a dry weight basis. The lucerne hay diet was higher in fibre content (31 as against 15 per cent) and lower in protein (36 as against 46 per cent). The protein and fibre composition of the two diets is shown below.

	Green lucerne diet	Lucerne hay diet
Fibre	84·3 gm per day	143·5 gm per day
Protein	196·0 gm per day	120·0 gm per day

Sampling

Samples of jugular blood (3 ml) were withdrawn from each sheep every morning at 8 a.m. before feeding.

Analytical

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

Results

Effect of diet and fasting on the live weights of the sheep

The purpose of the pre-fasting diet was to enable the animals to maintain a constant live weight. While this was achieved successfully by the lucerne hay diet, the initial green lucerne diet did not do so, the sheep in this group losing an average of 4 lb in the first week. An increase in the daily ration of green lucerne from 2,000 to 3,000 gm not only restored the weights of the animals within a week, but also enabled them to maintain these weights satisfactorily thereafter (Table 1).

TABLE 1.—*The effect of diet and starvation on the live weights of wethers*

Period	Weeks from start of experiment	Live weights lb					
		Green lucerne group			Lucerne hay group		
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Pre-starvation.....	0	93·0	75·5	83·0	85·5	95·5	95·0
	1	88·5	69·5	80·0	86·5	93·5	96·0
	2	92·5	74·5	83·5	85·5	93·0	96·0
	3	92·0	74·0	81·5	87·0	93·0	96·0
	4	93·0	74·5	83·5	86·5	93·5	96·5
End of starvation.....	6	86·5	69·0	76·5	76·5	81·0	81·5
Post-starvation.....	7	89·5	74·5	84·0	80·0	91·5	94·0
	8	92·5	75·5	84·0	82·5	92·5	94·0
	9	93·5	76·0	85·0	85·0	94·0	95·0
	10	93·0	76·0	84·0	85·0	95·0	95·0

Following the eight day period of starvation, striking differences were observed between the weight losses of the animals of the two groups. In the green lucerne group this loss averaged 6·3 lb per sheep (7·5 per cent) while in the lucerne hay group it was as high as 12·2 lb (13·3 per cent). Similar differences were observed during the post-fasting period. The animals on green lucerne regained their pre-fasting weights almost completely within a week despite capricious appetites, while those of the lucerne hay group displayed excellent appetites but only regained their pre-fasting weights after a period of three weeks although their rate of weight gain was greater than that of the green lucerne group. The differences in weight between the two groups during both fasting and post-fasting periods were analysed statistically (t test) and found to be highly significant ($P = 0{\cdot}01$).

The effect of diet and fasting on the blood sugar levels

During the pre-fasting period the average blood sugar level of the lucerne hay group was nearly twice as high (78·8 mg per cent) as that of the green lucerne group (40·7 mg per cent). During the fasting period, however, the position was reversed and the average blood sugar level of the former group was consistently lower than

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that of the latter (Table 2). At 120 hours of fasting the levels of both groups reached their minimum values 22·3 and 28·0 mg per cent respectively and thereafter tended to rise again to 37·5 and 47·8 mg per cent respectively at 192 hours. The differences in blood sugar levels between the two groups both during and after the fasting period were found to be highly significant ($P = 0·01$).

TABLE 2.—*The effect of diet and starvation on the blood sugar levels of wethers*

Days of starvation	Blood sugar levels in mg per cent							
	Green lucerne group				Lucerne hay group			
	No. 1	No. 2	No. 3	Ave.	No. 4	No. 5	No. 6	Ave.
0	35·0	45·0	42·5	40·7	77·0	80·5	78·5	78·7
1	34·5	28·5	50·5	37·8	31·5	32·5	28·5	30·8
2	21·5	34·0	36·0	30·5	31·0	22·0	25·5	26·2
3	41·0	33·0	41·0	38·3	28·5	29·5	27·0	28·3
4	34·5	29·5	33·0	32·3	30·0	28·0	30·0	29·3
5	26·0	24·0	34·0	28·0	19·5	25·0	22·5	22·3
6	28·5	32·5	36·0	32·3	31·5	37·5	28·5	32·5
7	26·0	28·0	33·0	29·0	25·5	27·5	27·0	26·7
8	29·5	34·5	40·5	34·8	28·5	37·5	31·0	32·3
Half ration	41·5	49·5	52·5	47·8	30·0	44·5	35·0	37·5
Full ration	49·5	52·5	44·5	48·8	39·5	46·0	51·0	45·5

The effect of diet and fasting on the total ketone body levels

In contrast to its effect on the blood sugar levels, the diet had no effect on either the pre-fasting or fasting total ketone body levels (Table 3). This was confirmed by a statistical examination of the results. The average pre-fasting levels of the three sheep on green lucerne and two out of three sheep on lucerne hay was 2·2 mg per cent, very close to the normal ketone body value of wethers (2·5 mg per cent) previously obtained by the author (Procos, 1961).

Starvation resulted in an increase of the mean ketone body level of these five sheep to a maximum of 4·9 mg per cent at 72 hours, after which it fluctuated between 4·3 and 4·6 mg per cent. It is noteworthy that the maximum value was attained 48 hours before the blood sugar had reached its minimum.

One sheep out of the six, however, reacted to starvation in a completely different way. This was wether No. 2 from the green lucerne group. Although the total ketone body level of this sheep was lower (1·3 mg per cent) than the average of the other five at the pre-fasting stage, nevertheless on fasting it rose above the mean values of the other sheep from the first day of starvation and continued to increase throughout the starvation period, reaching a maximum of 14·02 mg per cent at 192 hours. In all sheep the ketone body levels dropped within 48 hours of the resumption of feeding. This was particularly noticeable in No. 2 where the level fell from 14·02 to 2·19 mg per cent. Despite the long period of starvation, none of the sheep showed clinical symptoms of ketosis.

TABLE 3.—*The effect of fasting on the blood ketone bodies of wethers*

Days of fasting	Ketone bodies in mg per cent											
	Green lucerne group											
	Sheep No. 1				Sheep No. 2				Sheep No. 3			
	A	A–A	B–OH	TKB	A	A–A	B–OH	TKB	A	A–A	B–OH	TKB
0	0·12	0·86	1·00	1·98	0·12	1·19	0·00	1·31	0·96	1·82	0·32	3·10
1	1·08	1·05	0·03	2·16	0·96	2·48	0·29	3·73	0·96	1·99	1·57	4·52
2	0·48	0·83	2·00	3·31	1·08	1·87	3·02	5·97	0·60	0·55	2·50	3·65
3	1·08	0·98	2·33	4·30	2·04	3·21	2·65	7·90	0·84	1·62	1·57	4·03
4	1·20	1·09	2·01	4·30	2·04	2·06	3·15	7·25	1·20	1·42	2·51	5·13
5	2·04	1·24	1·81	5·09	3·48	3·35	1·58	8·41	1·56	1·39	2·08	5·03
6	1·80	0·82	1·18	3·80	4·80	3·75	0·80	9·35	2·52	2·40	1·40	6·32
7	2·16	0·79	0·73	3·68	5·90	4·07	0·19	10·16	2·04	2·71	0·10	4·85
8	1·56	0·90	3·33	5·79	8·00	4·02	2·00	14·02	0·48	1·65	2·33	4·46
Full ration 1 day.	0·00	0·00	3·81	3·81	0·60	0·88	0·71	2·19	0·00	0·16	1·22	1·38

A = acetone; A–A = acetoacetic acid; B–OH = β-hydroxybutyric acid; TKB = total ketone bodies

Days of fasting	Ketone Bodies in mg per cent											
	Lucerne hay group											
	Sheep No. 4				Sheep No. 5				Sheep No. 6			
	A	A–A	B–OH	TKB	A	A–A	B–OH	TKB	A	A–A	B–OH	TKB
0	0·60	1·37	0·00	1·97	0·48	1·49	0·12	2·09	0·36	1·28	0·22	1·86
1	0·60	1·20	0·29	2·09	0·84	1·62	0·20	2·66	0·60	1·86	0·20	2·66
2	0·60	0·88	1·98	3·46	0·84	1·29	3·54	5·66	1·20	1·09	2·02	4·31
3	1·08	1·05	2·84	4·97	1·44	1·35	3·08	5·87	1·32	1·63	2·33	5·28
4	1·56	1·39	0·00	2·95	1·20	1·09	3·53	5·82	1·32	1·47	0·98	3·77
5	1·56	2·05	4·15	4·15	1·56	2·70	0·05	4·31	1·20	1·75	1·57	4·52
6	1·68	2·30	0·40	4·38	2·16	2·22	1·08	5·46	1·20	1·26	1·12	3·58
7	1·80	2·30	0·00	4·10	1·80	1·81	3·47	7·08	1·68	1·27	0·48	3·43
8	0·96	1·50	0·54	3·00	0·60	1·86	2·06	4·52	0·36	1·93	1·32	3·61
Full ration 1 day.	0·12	0·04	0·73	0·89	0·00	0·16	0·22	0·38	0·00	0·16	0·12	0·28

A = acetone; A–A = acetoacetic acid; B–OH = β-hydroxybutyric acid; TKB = total ketone bodies

Days of fasting	Ketone bodies in mg per cent			
	Average of sheep No. 1, 3, 4, 5, 6			
	A	A–A	B–OH	TKB
0	0·50	1·36	0·33	2·19
1	0·82	1·54	0·46	2·82
2	0·74	0·93	2·41	4·08
3	1·15	1·31	2·43	4·89
4	1·30	1·30	1·81	4·41
5	1·58	1·83	1·21	4·62
6	1·87	1·80	1·04	4·71
7	1·90	1·78	0·96	4·64
8	0·79	1·57	1·92	4·28
Full ration 1 day	0·02	0·10	1·22	1·34

A = acetone; A–A = acetoacetic acid; B–OH = β-hydroxybutyric acid; TKB = total ketone bodies

The effect of fasting on the individual ketone bodies

On fasting, the β-hydroxybutyric acid fraction increased initially and then decreased, while the acetoacetic acid fraction showed a tendency to increase throughout. In all sheep, including No. 2, the pattern of change was similar. However, in the latter, these changes were consistently more pronounced especially as regards acetoacetic acid.

In contrast, the behaviour of the acetone fraction of sheep No. 2 was totally different from that of the rest of the animals. In the former, the rate of increase was found to be represented by a parabolic curve, while in the rest it was represented by a straight line up to the seventh day of fasting (Fig. 1). (The equations of the parabola and the straight line were calculated and found to be $Y = 0{\cdot}1X^2 + 0{\cdot}12X + 0{\cdot}402$ and $Y = 0{\cdot}21X + 0{\cdot}4$ respectively, where Y represents acetone concentration in mg per cent and X represents number of days of starvation.)

Discussion

Both pre-fasting diets used proved capable of maintaining the live weights of the animals and it can, therefore, be assumed that their calorific values were almost equal. They differed, however, with respect to protein and fibre content. The lucerne hay diet had a fibre to protein ratio of 1·2 while the green lucerne diet had a ratio of 0·4. This difference seemed to have a considerable influence on the pre-fasting blood sugar levels. Thus the animals fed the high fibre diet (lucerne hay) had blood sugar levels (78·7 mg per cent) which were almost twice as high as those (40·7 mg per cent) of the animals fed the high protein diet (green lucerne). This tendency for certain diets to influence the blood sugar of sheep has also been observed by Sampson & Boley (1940), who obtained similar high blood sugar values (69 mg per cent) by feeding alfalfa hay *ad lib.* supplemented with shelled corn (2 lb), while lesser amounts of hay resulted in lower blood sugar values.

On starvation the animals of the lucerne hay group lost on an average almost twice as much weight (12·2 lb) as those of the green lucerne group (6·3 lb). These differential losses were in all probability another consequence of the difference in diets. Since it is now established that during starvation fat is catabolised in preference to protein (Cumming & Morrison, 1960) and in view of the facile conversion of carbohydrate to fat, it seems reasonable to assume that the diet high in carbohydrate would be conducive to a higher loss of weight than the one high in protein.

During the starvation period the animals displayed a mild hypoglycemia. The minimum blood sugar values encountered were 28 mg per cent for the sheep of the green lucerne group and 22·3 mg per cent for those of the lucerne hay group. Both these values are lower than that (30 mg per cent) mentioned by Reid & Hogan (1959) as the expected blood sugar value for fasting non-pregnant animals.

It is now accepted that in the ruminant, only half of the daily glucose requirements is supplied by the propionic and in part by the acetic acid produced in the rumen during the digestion of food by the ruminal organisms. The other half is supplied by the conversion of protein to sugar (gluconeogenesis) (Lindsay, 1959). When, as in starvation, the first source of glucose is absent, the fasting animal will make increasing use of gluconeogenesis to supplement its carbohydrate reserves. In the present experiment this was indicated by the increase in blood sugar levels which occurred during the latter part of the starvation period.

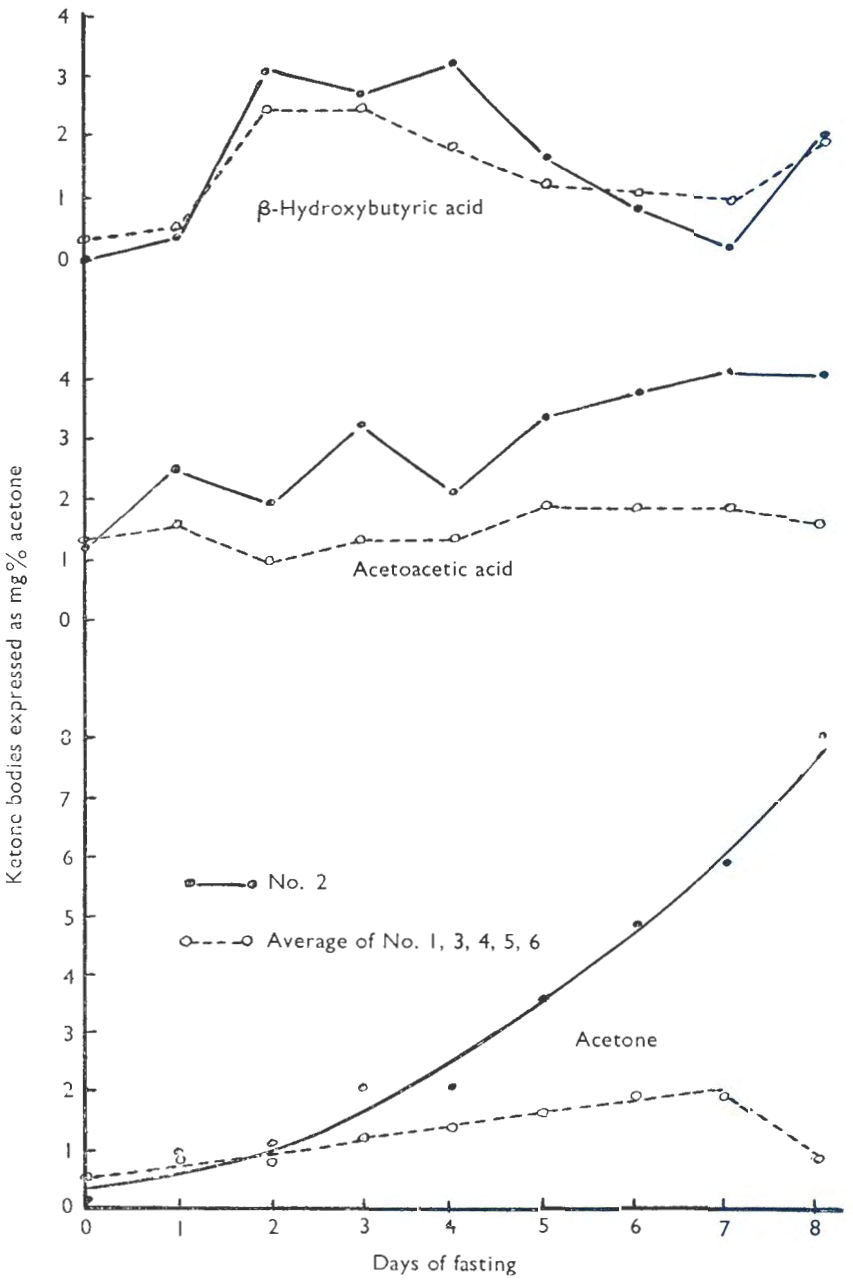

FIG. 1.—The variation of acetone, acetoacetic and β-hydroxybutyric acids during fasting.

The differences in blood sugar levels observed between the animals of the two groups during fasting, were in all probability due to the differences in protein content of the pre-fasting diets. It has been previously shown that a diet high in protein can lead to the maintenance of high glycogen reserves during fasting (Mirski, Rosenbaum, Stein & Wertheimer, 1938) and hence higher fasting blood sugar levels.

The total ketone body levels of the experimental animals rose during fasting from 2·2 to an average maximum value of 4·9 mg per cent. Similar maximum levels (4·7 mg per cent) were obtained by Sampson & Boley (1940) for non-pregnant ewes following a fasting period of equal length. These values are well below the levels (greater than 10 mg per cent) attained by sheep exhibiting clinical symptoms of pregnancy disease (Procos, unpublished).

In contrast, sheep No. 2 behaved in a different way, its maximum ketone body level reaching a value of 14 mg per cent. The reason for this abnormal behaviour is not clear especially in view of the fact that the fasting weights and blood sugar levels of this particular animal, were not greatly different from those of the other sheep in its group. It is, however, possible that food deprivation constituted a greater psychological stress to this animal.

Of the individual ketone bodies, both acetoacetic acid and acetone increased during the fasting period while β-hydroxybutyric acid first increased and later decreased. In sheep No. 2 these changes were of a similar nature but far more pronounced particularly as regards the first two fractions.

Acetoacetic acid is considered to be the parent ketone body substance giving rise to acetone by spontaneous decarboxylation and to β-hydroxybutyric acid by enzymic reduction mediated via β-hydroxybutyric dehydrogenase with reduced diphosphopyridine nucleotide (DPNH) as the hydrogen donor (Baldwin, 1957). The fact that in the present studies the increase in acetone occurred at a time when the concentration of β-hydroxybutyric acid was decreasing is an indication that acetoacetic acid was giving rise to increased quantities of the former at the expense of the latter. This decrease in β-hydroxybutyric acid production in the presence of increasing quantities of acetoacetate was probably due to a decreased supply of DPNH (Kulka, Krebs & Eggleston, 1961), although in the light of present knowledge no explanation can be offered for this decrease.

Conclusion

Prolonged starvation of wethers has been found to result in a mild hypoglycemia (average blood sugar level 25 mg per cent) and a slight ketonemia (average ketone body level 4·9 mg per cent) the only exception being wether No. 2 which developed a true but mild ketosis (maximum ketone body level 14 mg per cent). All the animals, including No. 2 displayed no visible clinical symptoms throughout the starvation period. It would thus appear that an eight day period of fasting is not capable of inducing a true hypoglycemic ketosis in normal, non-pregnant, non-lactating sheep.

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