Onderstepoort Journal of Veterinary Research,
Volume 28, Number 4, May, 1961.
The Government Printer, Pretoria.

OVINE KETOSIS I: THE NORMAL KETONE BODY VALUES

J. PROCOS, Onderstepoort Laboratory

INTRODUCTION

" Ketosis " is a term indicating the presence of greater than normal amounts of " ketone bodies "—acetone, acetoacetic acid and β-hydroxybutyric acid—in the blood.

Ketosis generally occurs following a variety of metabolic disturbances such as starvation, where carbohydrate is in short supply, and pancreatic diabetes, where carbohydrate utilisation is impaired. Ruminant animals are particularly susceptible to ketosis of the hypoglycaemic type such as the lactation ketosis of cows and pregnancy disease of sheep.

Although much work has been done in the past in attempts to establish the factors responsible for the ketosis of ruminants, the interpretation of the results has been hampered considerably by the lack of data on blood ketone levels of clinically healthy animals of various breeds and classes. For these reasons it was decided to make a detailed survey of the blood ketone body levels in healthy Merino ewes and wethers as a preliminary to more detailed studies on the carbohydrate and fat metabolism of these animals. A limited number of blood sugar determinations was also made during this experiment.

EXPERIMENTAL

The experiment was divided into two parts which lasted 21 and 68 days respectively (23 Nov. 1956–14 Dec. 1956; 21 Jan. 1957–1 Apr. 1957).

Animals

During the first part of the experiment only six non-pregnant Merino ewes were used while in the second part six Merino wethers were also included. Both ewes and wethers were good-conditioned adult animals, the former being particularly selected for the regularity with which they came into oestrus. The two groups of animals were maintained separately in open camps with young trees as the only shelter.

Diet

Each animal received approximately 350 gm. of concentrates daily together with good quality lucerne hay *ad lib.* The concentrates consisted of a mixture of oats (60 per cent), wheat bran (20 per cent), crushed maize (13 per cent), groundnut meal (5 per cent), salt (1 per cent) and lime (1 per cent). The animals were fed once daily (9 a.m.) from common troughs and had free access to water.

Received for publication on 25 September, 1960.—Editor.

818504—5

Determination of oestrus

Oestrus was determined twice daily (8·30 a.m. and 2 p.m.) with the aid of two vasectomised rams.

Sampling

Samples of jugular blood (2 ml.) were taken from each sheep every morning at 8 a.m. before feeding. Ketone body concentrations were estimated throughout the experiment while blood sugar was determined only during the first part.

Analytical

(*a*) *Ketone bodies.*—Total blood ketone bodies were estimated by the method of Thin & Robertson (1952). However, in order to increase the sensitivity over the lower range of ketone body concentrations (0–10 mg. per 100 ml. of blood) both standards and unknowns were diluted 1: 2 (1 ml. coloured solution to 1 ml. water), and read against blanks which were diluted 1: 9 (1 ml. coloured solution to 8 ml. water). In this way a calibration curve was obtained in which the normal ketone body values gave a reading in the optimum range of the optical density scale (0·100–0·300). The colour intensity was read on a Zeiss Opton spectrophotometer at 495 mμ using 2 mm. cells.

(*b*) *Blood sugar.*—Blood sugar was determined using Lehman & Silk's (1952) modification of the Folin & Wu (1920) method.

Meteorological data

The data was obtained from the official meteorological station situated at Onderstepoort and consisted of the following daily observations: rainfall (mm.), relative humidity (per cent) and the maximum and minimum temperatures. In addition to the above, the daily *temperature drop* (T.D.*) was calculated; this is the drop in temperature from the maximum of the previous afternoon to the minimum in the early hours of the morning on which sampling took place.

Results

Effect of oestrus on ketone body concentrations

In order to determine the effect of oestrus on the ketone body levels of the ewes, use was made of the values obtained over a 60 day period (second part of the experiment). Of the 360 values 331 corresponded to periods of anoestrus, while the remaining 29 were those of the ewes at oestrus. The mean ketone body value at oestrus was found to be 1·32 mg. per cent while during anoestrus the corresponding value was 2·08 mg. per cent.

The difference was tested statistically by the t-test and proved to be highly significant ($P = 0·01$).

* The term *temperature drop* has been adopted in consultation with Mr. A. J. Dreyer of the Weather Bureau, Pretoria, as being the most appropriate.

Effect of sex on ketone body concentrations

The means of the ketone body values obtained during the same 60-day period were estimated separately for ewes and wethers. However, in view of the previous findings, values obtained during days when one or more of the ewes were in oestrus were excluded from the calculations in both groups of animals.

The distribution of ketone body values in ewes and wethers is shown in Table 1. The mean ketone body concentrations were found to be 2·18 mg. per cent (204 determinations) and 2·73 mg. per cent (204 determinations) for ewes and wethers respectively. The difference was again tested statistically by means of the t-test and proved to be highly significant ($P = 0·001$).

Effect of weather on ketone body concentrations

(i) *Temperature drop.*—The range of maximum temperatures encountered in this study was 33–17·2°C for the first part of the experiment (late spring to early summer) and 33·5–22·3°C for the second (late summer to early autumn). The range of minimum temperatures was 8·2–16·4°C and 10·4–18·1°C, respectively. The temperature drops during the first period, ranged from 4·9–20·2°C with a mean of 12·8°C, while during the second period the range was 6·1–22·1°C with a mean of 15·5°C. On days when measurements were taken, however, the actual range of temperature drops was 7–20°C. The overall mean temperature drop for the whole experiment was 14·7°C which is close to the mean yearly temperature drop of the Pretoria region (13·7°C) over the past five years.

The average ketone body values for each day were calculated separately for wethers and ewes, the latter only during periods of anoestrus (Table 2). Inspection of the results obtained during the first part of this experiment suggested the existence of a positive relationship between ketone body levels and their corresponding temperature drops. Thus with a view to analysing the results statistically the temperature drops, in each group of animals, were divided into two sections (7–13·9°C and 14–20°C), plotted against their corresponding ketone body values and straight lines fitted through the points by the method of least squares. In each case the correlation coefficient was calculated and the following results obtained:—

In the upper range of temperature drops (14–20°C) the positive relationship between these two factors proved in fact to be highly significant for both ewes and wethers (correlation coefficients 0·6203 and 0·7611, $P = 0·01$ and 0·001 respectively). The rates of increase of ketone body levels per degree of temperature drop were found to be 0·24 and 0·31 mg. per cent for ewes and wethers respectively. However, a t-test of the difference between the regression coefficients of the two fitted lines proved that these rates of increase were not, significantly different from each other.

In the lower range of temperature drops (7–13·9°C) the situation is quite different. The straight lines run parallel to the x-axis cutting the y-axis at 1·77 and 2·15 mg. per cent ketone body levels for ewes and wethers respectively, and there is no significant relationship between this factor and temperature drops. For purposes of comparison, the two pairs of straight lines obtained above have been combined, and are presented in Fig. 2.

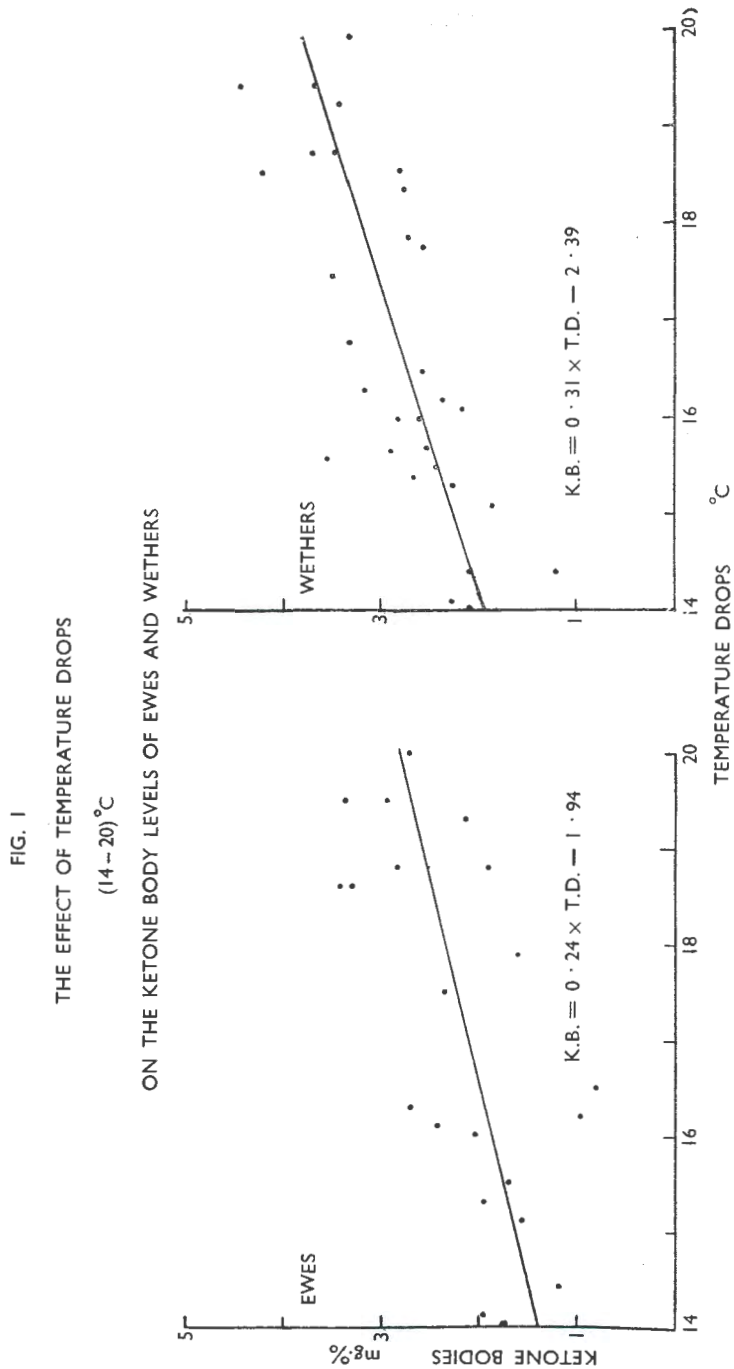
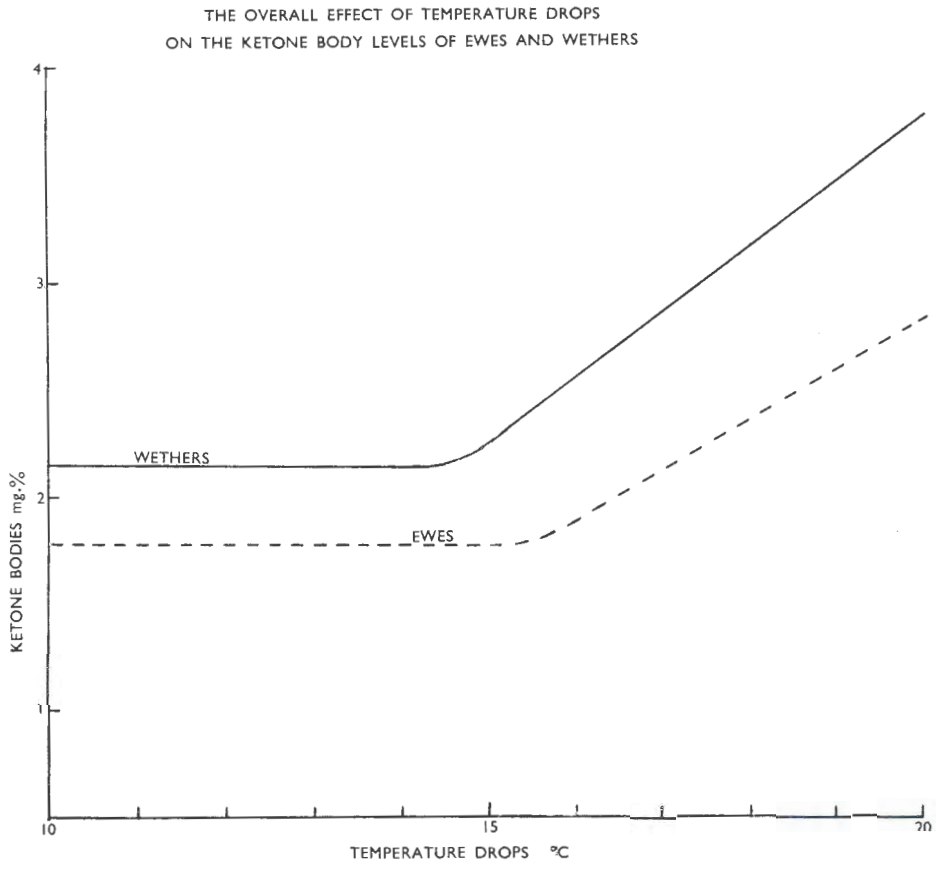


FIG. 1

THE EFFECT OF TEMPERATURE DROPS (14 – 20) °C ON THE KETONE BODY LEVELS OF EWES AND WETHERS

Fig. 2



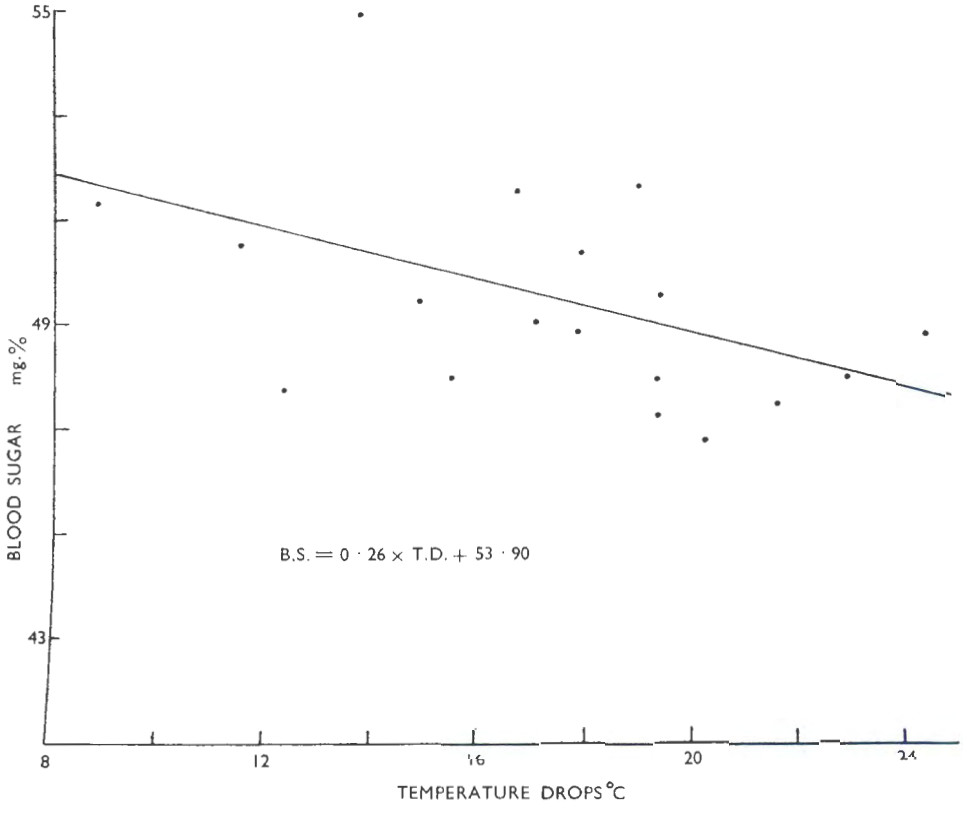
(ii) and (iii) *Rainfall and humidity*.—When rainfall and humidity were divorced from temperature drop no significant correlation was found to exist between these two factors and ketone body values in either ewes or wethers.

Effect of temperature drops on the blood sugar levels of ewes

The effect of temperature drops on the blood sugar levels of the ewes was similarly analysed, this time using the values obtained during the first part of the experiment. However, in view of the small number of determinations (108), no attempt was made to separate the temperature drops into two sections as in the case of the ketone bodies. The results in Fig. 3 show that a negative correlation (correlation coefficient $-0{\cdot}4923$) exists between temperature drops and blood sugar which is significant at $P = 0{\cdot}05$. The rate of decrease in blood sugar per degree of temperature drop was found to be 0·26 mg. per cent.

Fig. 3

EFFECT OF TEMPERATURE DROPS ON THE BLOOD SUGAR LEVELS OF EWES



The normal ketone body values of ewes and wethers

The results above show conclusively that the blood ketone body levels of clinically healthy sheep are influenced by sex, large drops in temperature and, in the case of the ewes, by oestrus. These factors must, therefore, be taken into consideration when calculating normal ketone body values. In the present study two separate mean normal values were obtained for each group of animals. The first was the ordinary mean value which embraces all the ketone body values obtained during this experiment, while the second one has been called the *absolute mean value* and includes only those ketone body values corresponding to temperature drops below 14°C and in addition, in the case of the ewes, those values obtained during periods of anoestrus. The two sets of normal values together with their standard deviations and ranges are shown in Table 3.

Discussion

Sex and oestrus

In the present study highly significant differences were shown to exist between the ketone body levels of ewes and wethers. In addition the ketone body levels of ewes at oestrus were found to be significantly lower than the corresponding values obtained during the periods of anoestrus. While considerable amount of work has been done in the past on the effect of sex on the metabolism at the enzymic level and to a lesser extent on the carbohydrate metabolism, comparatively few studies are available of its effect on the ketone body levels. In the case of monkeys and cats Friedemann (1942) and Chamberlin, Furgason & Hall (1937) had previously shown that the males had a higher rate of urinary ketone body excretion than the females. Castration also has been found to increase the ketone body levels in the rat (Tronci, 1938).

The effect of oestrogen administration on the carbohydrate metabolism has been extensively studied and found invariably to lead to an increase in blood sugar and liver glycogen levels (Zunz & La Barre, 1939; Janes & Nelson, 1940). Oestrus has been similarly observed to result in a rise in blood sugar levels in heifers (Hewitt, 1930; Hodgson, Riddel & Hughes, 1932). However, the effect of oestrus on the ketone body levels has not been previously studied. Our results indicate that in view of the inverse relationship existing between ketone body and blood sugar levels, the fall in the levels of the former is in full agreement with the findings of the above research workers.

Temperature

A most important finding of the present study has been the fact that sudden drops in temperature constitute an important stress factor for sheep. The result of this is an increase in ketone bodies and a decrease of sugar in the blood. Results of a similar nature have recently been reported for dairy cows by Van Soest (1960).

Sudden exposure to cold has been recognised as a form of stress resulting in an increased rate of hepatic ketogenesis in humans (Sargent & Consolazio, 1951) and rats (Scott & Engel, 1953). This increase in ketone body production is probably due to the increased utilisation of energy reported by Sellers (1957) and the concomitant decrease in depot fat reserves demonstrated recently by Campbell, Green, Schönbaum & Socol (1960). On the other hand the effect of cold exposure on the carbohydrate metabolism has been shown to result in a lowering of both blood sugar levels (Masson, 1941; Baker & Sellers, 1953) and liver glycogen reserves (Masoro & Felts, 1959).

Although it has been generally accepted that animals have a considerable capacity to adapt themselves to environmental changes, it is only recently that acclimitisation has been actually shown to occur in rats, resulting in the maintenance of normal blood sugar (Baker & Sellers, 1953) and glycogen levels (Felts & Masoro, 1959). In the present study it was clearly shown that the animals responded only to temperature drops greater than 14°C. If it is taken into consideration that the mean yearly temperature drop of the Pretoria region is approximately 14°C, it seems very likely that the sheep in this area have become acclimatised to temperature drops of this magnitude and consequently respond only when the temperature drop is greater.

It is interesting to note that, in sheep, occurrence of metabolic disorders such as pregnancy toxaemia and loss of appetite, have in the past been attributed to " sudden drops in temperature " (Dimock, 1930), " bad storms " (Roderick & Harshfield, 1932) and " sudden changes in the weather " (Sampson, 1947). It is evident from our present results that abnormally large changes in temperature constitute an additional stress which could precipitate the disease in animals predisposed to it by other factors such as pregnancy and semi-starvation.

It is now clearly evident that the normal levels of blood ketone bodies in sheep must be considered in relation to the sex and sex cycle of the animals and that in addition the temperature fluctuations to which the animals are exposed must also be taken into account. The absolute mean values for wethers was found to be 2·15 mg. per cent with a range of 0–5·05 mg. per cent, while that of the ewes was 1·77 mg. per cent with a range of 0–3·85 mg. per cent. Finally the combined absolute mean value 1·93mg. per cent was in excellent agreement with the means of baseline figures obtained from both ewes and wethers by Goetsh (1957) 1·96 mg. per cent, Clark & Malan (1956) 1·80 mg. per cent and Williams & Christian (1959) 1·78 mg. per cent.

Summary

The ketone body levels of ewes at oestrus have been shown to be significantly lower than during anoestrus.

The ketone levels of wethers have been found to be significantly higher than that of ewes.

A positive relationship has been established between temperature drops of more than 14°C. and ketone body levels.

A negative relationship has been established between temperature drops and blood sugar levels.

The normal ketone body values of ewes and wethers together with their respective standard deviations and ranges are presented.

Acknowledgements

This paper is published with the approval of the Director of Veterinary Services, Onderstepoort, and forms part of a thesis to be presented to the University of South Africa for the degree of Doctor of Philosophy. A report of this work was presented before the South African Chemical Society (Medical Biochemistry Section) at its annual convention in July, 1959.

The author wishes to thank Drs. F. M. C. Gilchrist and H. M. Schwartz for their help and encouragement during this work and their helpful criticisms of the manuscript. Thanks are also due to Miss A. Scholz and Mr. R. J. J. Briel for technical assistance and to Messrs. C. M. Havenga and S. G. Reinach of the Faculty of Agriculture, University of Pretoria, for their assistance with the statistical analyses and the presentation of the results.

REFERENCES

BAKER, D. G. & SELLERS, E. A. (1953). Carbohydrate metabolism in the rat exposed to a low environmental temperature. *Am. J. Physiol.* Vol 174, p. 459.

CAMPBELL, J., GREEN, G. R., SCHÖNBAUM, E. & SOCOL, H. (1960). Effects of exposure to cold and of diet on coenzyme A levels in tissues. *Can. J. Biochem. and Physiol.* Vol. 38, p. 175.
CHAMBERLIN, P. E., FURGASON, W. H. & HALL, V. E. (1937). Ketone body excretion in the cat: Sex difference, and influence of diet, fasting, castration and male hormone administration. *J. Biol. Chem.* Vol. 121, p. 599.
CLARK, R. & MALAN, J. R. (1956). Alterations in the blood sugar and ketone levels caused by dosing acetate, propionate and butyrate into the rumen of sheep. *Onderstepoort J. Vet. Res.* Vol. 27, p. 101.

DIMOCK, W. W. (1930). Acidosis of pregnant ewes. *Vet. Alumni Quart. College Vet. Med. Ohio State University.* Vol. 17, p. 149.

FELTS, J. M. & MASORO, E. J. (1959). Effects of cold acclimation on hepatic carbohydrate and lipid metabolism. *Am. J. Physiol.* Vol. 197, p. 34.
FOLIN, O. & WU, H. (1920). A simplified and improved method for determination of sugar. *J. Biol. Chem.* Vol. 41, p. 367.
FRIEDEMANN, T. E. (1942). Ketosis in primates. *J. Biol. Chem.* Vol. 142, p. 635.

GOETSH, G. D. (1957). Studies on the production and treatment of experimental ketosis of ruminants. Thesis, Purdue Univ.

HEWITT, E. A. (1930). The blood sugar level of the bovine. *J. Am. Vet. Med. Assoc.* Vol. 77, p. 362.
HODGSON, R. E., RIDDEL, W. H. & HUGHES, J. S. (1932). Factors influencing the blood sugar level of dairy cattle. *J. Agric. Res.* Vol. 44, p. 357.

JANES, R. G. & NELSON, W. O. (1940). Effect of stilboestrol on certain phases of carbohydrate metabolism. *Proc. Soc. Exp. Biol. & Med.* Vol. 43, p. 340.

LEHMAN, H. & SILK, E. (1952). The prevention of colour fading in the Folin and Wu estimation of the blood sugar. *Biochem. J.* Vol. 50, XXXI.

MASORO, E. J. & FELTS, J. M. (1959). A biochemical mechanism for the depression in hepatic acetate oxidation in fasted, cold-exposed rats. *J. Biol. Chem.* Vol. 234, p. 198.
MASSON, G. (1941). Action of nocuous agents on insulin and adrenaline sensitivity and on glucose tolerance. *Endocrinology.* Vol. 29, p. 453.

RODERICK, L. M. & HARSHFIELD, G. S. (1932). Pregnancy disease of sheep. *Bulletin* 261, *N. Dakota Agric. Exp. Station.*
SAMPSON, J. (1947). Ketosis in domestic animals. *Bulletin* 524, *Univ. of Illinois Agric. Exp. Station.*

SARGENT, F. II & CONSOLAZIO, C. F. (1951). Stress and ketone body metabolism. *Science* Vol. 113, p. 631.
SCOTT, J. L. JR. & ENGEL, F. L. (1953). The influence of the adrenal cortex and cold stress on fasting ketosis in the rat. *Endocrinology.* Vol. 53, p. 410.
SELLERS, E. A. (1957). Effect of cold exposure and fasting on pathways of hepatic glucose oxidation. *Rev. Can. Biol.* Vol. 16, p. 175.

THIN, CHRISTIAN & ROBERTSON, A. (1952). The estimation of acetone bodies. *Biochem. J.* Vol. 51, p. 218.

TRONCI, L. (1938). The influence of folliculin on the fasting ketonuria in normal and castrate rats *Sperimentale.* Vol. 92, p. 594.

VAN SOEST, P. J. (1960). United States Dep. of Agriculture, Report No. 1722–60.

WILLIAMS, V. J. & CHRISTIAN, K. R. (1959). Blood chemistry and haematology in sheep in a sudden decrease in food intake. *N.Z.J. Agric. Res.* Vol. 2, p. 677.

ZUNZ, E. & LA BARRE, J. (1939). The effects of sexual hormones on the regulation of glucemia. *Arch. Int. de Physiol. (Liege).* Vol. 48, p. 287.

TABLE 1.—*The effect of sex on the distribution of the total ketone body values*

Range of ketone body concentrations in mg. per cent	Percentage of observations	
	Ewes	Wethers
0·00–1·00	23	9
1·05–2·00	28	24
2·05–3·00	29	31
3·05–4·00	12	19
4·05–5·00	6	14
5·05–6·00	1	2
6·05–7·00	0	1

TABLE 2.—*The average ketone body values of six ewes and six wethers together with their corresponding temperature drops*

Temperature drops in °C	Ewes	Wethers
	Ketone bodies in mg. per cent.	
7·0	2·01	2·11
7·5	—	2·13
9·9	0·82	0·71
10·3	0·82	1·18
10·5	2·49	3·54
11·6	2·14	3·15
11·9	1·99	1·68
12·0	2·17	2·47
12·1	2·29	2·35
12·1	0·84	1·45
13·5	0·58	2·74
13·5	2·24	0·94
13·7	2·12	2·89
13·9	2·36	2·87
13·9	2·03	2·05
14·0	1·76	2·08
14·1	1·94	2·27
14·4	1·18	2·07
14·4	—	1·20
15·1	1·54	1·85
15·3	1·95	2·24
15·4	—	2·64
15·5	1·70	2·43
15·6	—	3·53
15·7	—	2·51
16·0	2·03	2·58

TABLE 2 (*continued*)

Temperature drops in °C	Ewes	Wethers
	Ketone bodies in mg. per cent.	
16·0	—	2·79
16·1	2·42	2·15
16·2	0·93	2·34
16·3	2·69	3·15
16·5	0·79	2·56
16·8	—	3·30
17·5	—	3·47
17·8	—	2·54
17·9	1·58	2·71
18·4	—	2·74
18·6	3·41	4·21
18·6	3·28	2·78
18·8	2·83	3·45
18·8	1·89	3·68
19·3	2·13	3·39
19·5	2·93	3·65
19·5	3·36	4·42
20·0	2·71	3·29

TABLE 3.—*The normal and* absolutely normal *total ketone body values of non-pregnant ewes and wethers*

Animals	Classification	Number of observations	Mean of total ketone body values mg. per cent	S.D.	Range
Ewes.....	Normal.....	204	2·00	±1·01	0–5·50
	Abs. Normal.	84	1·77	±0·93	0–3·85
Wethers.....	Normal.....	264	2·54	±1·24	0–6·15
	Abs. Normal.	90	2·15	±1·06	0–5·05
Ewes and Wethers	Normal.....	468	2·27	±1·18	—
	Abs. Normal.	174	1·93	±1·01	—