A STUDY OF

"KETOSIS IN THE RUMINANT"

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I N D E X.

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I. INTRODUCTION.

INTRODUCTION.

In the ruminant species ketosis is a common disorder of considerable economic importance, as the condition occurs most frequently in the dairy cow with a high milk production record, and in the ewe carrying more than one lamb. The result, in the case of the cow, is a marked deterioration in the general condition of the animal, with a considerable fall in milk yield, to the detriment of any recording practised. In addition the milk produced is often badly tainted. In the ewe the results are, in general, more drastic, as death is likely to occur; if parturition takes place the ewe generally recovers, but the young may be unthrifty.

Post parturient dyspepsia of cattle (bovine ketosis) and pregnancy toxaemia are characterised by an abnormal accumulation of acetone bodies in the blood, milk and urine. Many investigations have been carried out on the clinical aspects of ketosis, and a confusing number and variety of substances have been suggested as curative agents, none of which has been consistently effective. On purely theoretical bases, an equally large number of hypotheses have been put forward at different times regarding the aetiology. Before a comprehensive theory can be advanced, or the ideal cure found, the processes of ruminant metabolism must be further elucidated. It is obviously desirable to know the origin/

origin and fate of acetone bodies in the normal ruminant if their significance in disease is to be fully determined. With this in mind a series of experiments was planned to study:-

1. Experimental fasting ketosis in cows and ewes.
2. The metabolism of the acetone bodies in the two species.

Most of the work on acetone body formation and metabolism has been carried out on the rat, rabbit, dog and man, and the results obtained have been applied to all mammals irrespective of whether they are single stomached or ruminants. As the digestive system of a ruminant is entirely different from that of a dog, for example, it is unsafe to assume that the endogenous metabolism in the two species is completely similar.

The general belief is that acetone bodies (viz., acetone, acetoacetic acid and B-hydroxybutyric acid) are formed normally in the liver during the metabolism of fat and certain amino acids, either as intermediate products in the breakdown of fatty acid chains, or as side products from intermediary metabolites. Acetoacetic acid appears to be the parent substance, B-hydroxybutyric acid being formed by reduction. Under the influence of the widely distributed/

distributed B-hydroxybutyric dehydrogenase and Coenzyme I. acetoacetic acid and B-hydroxybutyric acid are freely interconvertable. Acetone is formed from acetoacetic acid by spontaneous decarboxylation, which is believed to take place at an appreciable speed under physiological conditions of temperature and pH. (Baldwin, 1949).

The acetone bodies are formed by the liver, but utilised by the muscles for energy purposes, being metabolised by means of the tricarboxylic acid cycle to carbon dioxide and water. (Barnes and Gurin, 1948). During periods of carbohydrate deprivation, such as starvation, when the glycogen reserves of the liver are exhausted, or diabetes, when the power of the liver to store glycogen is impaired, excessive quantities of fat are metabolised to supply the energy demands. As a result, the production of the acetone bodies increases till a point is reached when the rate of production exceeds the rate of catabolism, and a ketonaemia results. To counteract this large quantities are excreted in the urine,(ketonuria).

It has been shown that the liver is the chief, if not the only, source of acetoacetic acid in the single stomached animal. The investigations of Pennington (1951), into the absorption of volatile fatty/

fatty acids from the rumen, have shown that rumen epithelial tissue is capable of converting butyric acid into acetone and acetoacetic acid, and traces of B-hydrobutyric acid. This production of acetone bodies by tissues other than the liver is very unusual and may play a significant part in ruminant metabolism.

As well as the common acetone bodies, viz., acetone, acetoacetic acid and B-hydroxybutyric acid, we have found appreciable quantities of iso-propanol in the body fluids of cows and sheep affected with ketosis, but none in normal healthy animals. (Robertson et al. 1951). This factor may possibly play an important part in bovine ketosis and pregnancy toxaemia, so, in the following experiments, the origin and fate of iso-propanol has been studied with that of the acetone bodies. In this investigation the terms "acetone bodies" and "total acetone bodies" are used to include acetone, acetoacetic acid, B-hydroxybutyric acid and iso-propanol.

II. METHODS OF ESTIMATION.

INTRODUCTION.

In a study of the pathogenesis of bovine ketosis it was felt desirable to be able to follow in some detail the changes in all three ketones, viz., acetone, acetoacetic acid and B-hydroxybutyric acid, which have been shown by various workers to occur in excessive quantities in blood, milk and urine during the course of that disorder. Hitherto little or no attempt has been made at complete differentiation as both acetone and acetoacetic acid are usually determined in the one fraction. Moreover, iso-propanol which we have found (Robertson et al., 1950) to be present in this disease and in pregnancy toxaemia of ewes, has been unsuspected hitherto in such conditions and as it is oxidised to acetone by chromic acid, its presence will undoubtedly have influenced the estimation of total ketones by various methods.

The accurate determination of free acetone in the presence of acetoacetic acid is by no means easy. Van Slyke's method, using Denige's reagent (Van Slyke, 1917 and 1929) which has frequently been utilised in ketosis studies, cannot be used to estimate free acetone; in addition it is not very sensitive as we have found that no precipitate is formed with small amounts of acetone bodies of the order of 5 mg. per/

per 100 ml. or less. Moreover, using this method, iso-propanol appears to be oxidised to acetone partly in the acetone plus acetoacetic acid fraction, and partly in the B-hydroxybutyric acid fraction, so making the accurate determination of the individual acetone bodies impossible.

Attempts at using the distillation methods of Shaffer and Marriott (1915) and Behr (1928 and 1940) for free acetone by distillation under reduced pressure, or by blowing a fine stream of air through the solution at room temperature, in order to prevent acetoacetic acid decomposing, gave poor results, as, with quantities such as were likely to be present in biological fluids, only a small proportion of the acetone could be recovered. The method of Werch (1940 and 1941) utilising diffusion into Nessler's solution in Conway micro-diffusion units, though providing a very delicate qualitative test, did not give accurate quantitative results; the time factor for the appearance of the precipitate varied somewhat and the precipitates could not be estimated gravimetrically with any degree of success.

Behr and Benedict (1926) determined acetone, preformed, from acetoacetic acid and from B-hydroxybutyric acid, colorimetrically by its reaction with salicylic/

salicylic aldehyde in alkaline solution, the coloured product formed being dihydroxy-dibenzene-acetone. Seifert (1948) made use of the same colour reaction in the micro-estimation of acetone bodies in blood; the oxidation reactions and diffusion of the acetone formed into an alkaline solution of salicylic aldehyde, were carried out at the same time, in a small container similar to a Widmark flask. Greenberg and Lester (1944) employed a small micro-refluxing apparatus to prevent loss of acetone during oxidation. Their method of estimation involved the production of a hydrazone with 2-4-dinitro phenyl hydrazine, which they separated by extraction with carbon tetrachloride and estimated colorimetrically.

None of these methods proved entirely satisfactory as very few were designed for the estimation of free acetone, and none took iso-propanol into account. The necessity for improvement was obvious and the following method was, therefore, devised, combining with some modification the oxidation technique of Greenberg and Lester (1944), the diffusion method of Werch (1940 and 1941) and Seifert (1948) and Behr and Benedict's colorimetric technique (1926). It depends on the development of an orange to red colouration when an alkaline solution/

solution of salicylic aldehyde is left in the presence of acetone, the depth of colour formed being directly proportional to the amount of acetone present. This can be used to estimate free acetone, the acetone formed by hydrolysis of acetoacetic acid, and that formed by chromic acid oxidation of B-hydroxy: butyric acid and of iso-propanol.

METHOD.

Apparatus:

Micro-refluxing apparatus as used by Greenberg and Lester (1944).

Conway micro-diffusion units.

Reagents:

0.15N (approx.) barium hydroxide.
2.5% (approx.) zinc sulphate ($ZnSO_4.7H_2O$).
} prepared according to Greenberg and Lester (1944).

20N sulphuric acid.

10% potassium dichromate.

1.5% potassium dichromate in 15.6N sulphuric acid.

20% acetic acid.

4N potassium hydroxide.

Salicylic aldehyde, B.D.H. lab. reagent.

Colour Reagent:

8 ml. of 4N potassium hydroxide are added to 1 ml. salicylic aldehyde and the solution well mixed; 2 ml. are used for each estimation. This reagent tends/

tends to blacken when exposed to the air for several hours, and so a fresh supply must be made for each series of estimations.

Preparation of Standard Graphs.

Standard solutions of acetone are used, prepared according to Behr and Benedict (1926) containing from 0-10 mg. acetone per 100 ml. solution. 3 ml. of the standard solution are placed in the outer chamber of a Conway dish with a few drops of 20% acetic acid; 2 ml. of the colour reagent are placed in the inner chamber. The lid is put on firmly, after greasing the rim, and the dish is left for from half an hour to three hours in an incubator at 37°C., or any other standard time and temperature that is convenient; alternatively, it may be kept at room temperature overnight, when the maximum colour is obtained regardless of temperature. It should not be left standing, however, more than 20 hours as the colour reagent tends to blacken after this interval. A blank is run using water instead of the standard solution.

After the requisite time 0.5 ml. of the coloured solution in the central chamber is added to 2 ml. water in a test tube and the resulting solution read in the Spekker against the blank similarly prepared, using 2 ml. cells and a blue-green/

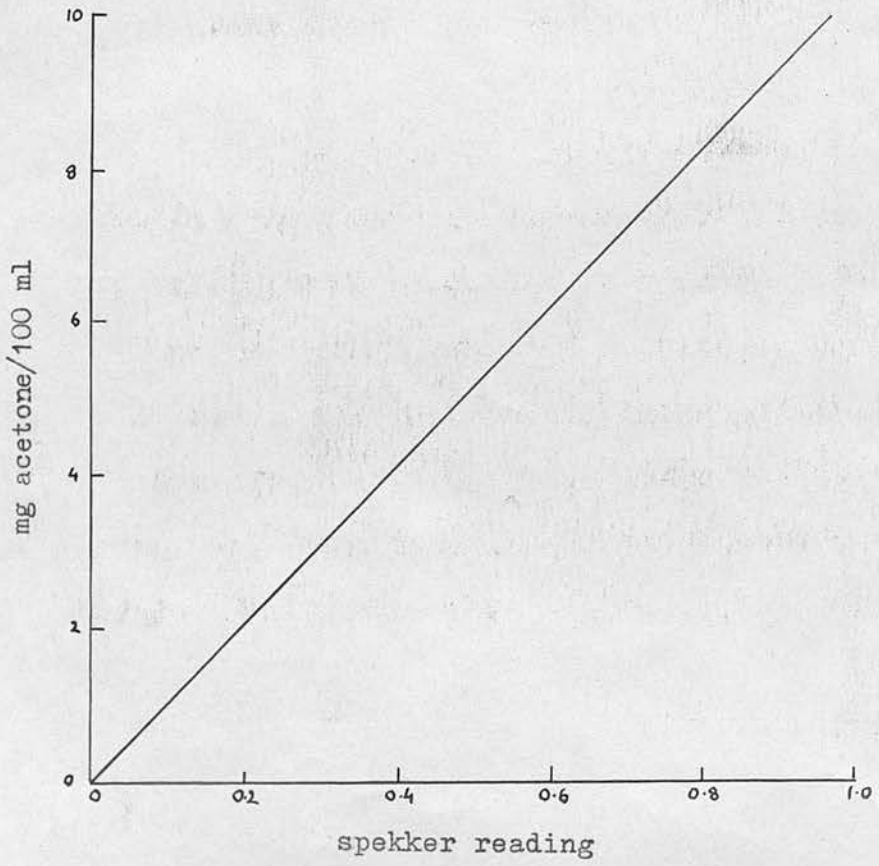
TABLE 1.

Reproducibility using standard solutions of acetone.

Time Interval.	Theoretical Result. mg./100ml.	Acetone Found. mg./100 ml.	Error. %
2 Hours.	7.92	7.80	- 1.5
2 Hours.	7.92	8.00	+ 1.0
Overnight.	7.92	7.70	- 2.8
Overnight.	7.92	8.10	+ 2.3
2 Hours.	1.98	1.90	- 4.0
2 Hours.	1.98	2.03	+ 2.5
Overnight.	1.98	2.00	+ 1.0
Overnight.	1.98	2.05	+ 3.5

FIGURE I.

A graph prepared by estimating standard solutions of acetone after 2 hours incubation.



green filter (Ilford 603).

Fig. 1. shows a graph prepared by estimating known amounts of acetone after 2 hours incubation. It will be seen that Beer's Law is strictly adhered to over the range 0-10 mgs. per 100 ml. From Table 1 it will be seen that results with standard acetone solutions are reproducible within ±4%.

Application to Biological Material.

I. Blood.

Protein precipitation: To 2 ml. distilled water in a centrifuge tube 1 ml. blood is added and the solution well mixed; 3 ml. barium hydroxide solution are then added, followed by 3 ml. zinc sulphate solution. The solution is well mixed, the tube stoppered and centrifuged. The clear supernatent liquid is used for estimating acetone, acetone plus acetoacetic acid and "Total Acetone Bodies".

(1) Free acetone.

3 ml. of the supernatent fluid are placed in the outer chamber of a greased Conway dish and the estimation carried out as above. Owing to dilution during precipitation and oxidation, it is necessary to multiply the results obtained from the standard graphs by the factor 9 to get the results in mg. acetone per 100 ml. of original solution.

Free/

TABLE 2.

Recovery of added acetone.

Acetone Added. mg./100 ml.	Acetone Found. mg./100 ml.	Blank. mg./100 ml.	Error. %
A. Standard acetone solution.			
7.92	7.80	0	- 1.5
7.92	8.00	0	+ 1.0
7.92	8.10	0	+ 2.3
B. Standard solutions in blood.			
7.92	8.01	0	+ 1.1
7.92	7.83	0	- 1.1
7.92	8.10	0	+ 2.3
15.84	16.20	0	+ 2.3
C. Standard solutions in blood (direct method).			
7.92	8.00	0	+ 1.0
7.92	8.10	0	+ 2.3
7.92	8.20	0	+ 3.5
15.84	15.50	0	- 2.1

Free acetone can also be estimated without precipitation of protein if so desired. Recovery experiments which were carried out using the complete technique of precipitation, etc., on standard solutions in water and blood showed the same limits of accuracy as in direct estimation of standard solutions (Table 2).

(2) Acetoacetic Acid.

Acetoacetic acid is determined by difference, the free acetone determined as above being subtracted from the acetone plus acetoacetic acid value, obtained as follows:

Acetone plus Acetoacetic Acid.

3 ml. of the supernatent solution are placed in the micro-refluxing apparatus and 1.1 ml. 20N sulphuric acid and a glass bead are added. The apparatus is shut firmly after applying a very thin film of grease to the ground-glass joint, the flow of water is started and the contents of the reflux tube boiled for 5 minutes. The apparatus is then cooled rapidly under running water, tipped to mix well, and 3 ml. of the solution are transferred to the outer chamber of a Conway dish for the determination of the acetone present. The factor in this case is 12.3.

Recovery/

TABLE 3.

Recovery of acetoacetic acid, expressed as acetone.

Amount Added. mg./100 ml.	Amount Found. mg./100 ml.	Blank. mg./100 ml.	Error. %
A. Standard solution.			
5.09	5.04	0	- 0.9
5.09	5.04	0	- 0.9
10.18	10.10	0	- 0.8
10.18	9.84	0	- 3.3
19.57	19.68	0	+ 0.6
19.57	19.68	0	+ 0.6
B. Standard solutions in blood.			
5.09	5.16	0	- 1.4
5.09	5.04	0	- 0.9
5.76	18.45	12.92	- 4.0
5.76	18.45	12.92	- 4.0
11.52	24.60	12.92	+ 1.4
11.52	24.60	12.92	+ 1.4

Recovery experiments were carried out as before using commercial grade ethyl acetoacetate purified by distillation under reduced pressure. As will be seen from Table 3 the results obtained came within $\pm$4% of the theoretical amounts.

(3) Iso-propanol.

Iso-propanol is oxidised to acetone under the conditions normally employed for estimating "Total Acetone Bodies" and so is included in this term. It can also be estimated separately using a modification of Friedmann's method (1938) in which the iso-propanol alone is oxidised to acetone. A Markham steam distillation apparatus is used in this procedure, except in the case of blood, as it is more convenient for small quantities, as well as being quicker and easier to clean between samples.

Reagents:

10% sodium tungstate solution.

20N sulphuric acid.

10% calcium hydroxide suspension.

Mercuric sulphate solution prepared according to Friedmann (1938).

Magnesium sulphate.

Potassium dichromate.

Method:/

Method:

50 ml. of blood are transferred to a large conical flask with 100 ml. distilled water, 10 ml. mercuric sulphate solution are added and the solution mixed, then 15 ml. sodium tungstate and a little wax to prevent frothing. The solution is well shaken and steam-distilled, about 100-200 ml. distillate being collected.

The distillate is washed into a round bottomed flask, 5 ml. mercuric sulphate solution added and excess calcium hydroxide suspension till the solution is alkaline. This solution is distilled directly into a conical flask, about 100 ml. distillate being collected. 10 ml. 20N sulphuric acid and excess potassium dichromate are added to the distillate and the flask, loosely corked, is either placed on a drying oven (about 50°C.) for 2 hours, or left overnight at room temperature.

The solution is then washed into a round bottomed flask and about 50 gms. magnesium sulphate added. The solution is distilled direct, about 100 ml. distillate being collected; this is made up to 100 ml. with distilled water in/

TABLE 4.

Recovery of iso-propanol, expressed as acetone.

Amount Added. mg./100 ml.	Micro-distillation.			Friedmann.		
	Amount Found. mg./100 ml.	Blank. mg./100 ml.	Error. %	Amount Found. mg./100 ml.	Blank. mg./100 ml.	Error. %
A. Standard solution.						
7.43	7.38	0	- 0.6	7.25	0	- 2.4
7.43	7.38	0	- 0.6	7.50	0	+ 0.9
7.43	7.38	0	- 0.6	7.25	0	- 2.4
B. Standard solution in blood.						
7.43	11.81	4.31	+ 0.9	7.50	0	+ 0.9
7.43	11.81	4.31	+ 0.9	7.25	0	- 2.4
7.43	11.44	4.31	- 4.0	7.25	0	- 2.4

in a graduated flask and the acetone content determined by placing 3 ml. of the resulting solution in the outer chamber of a Conway dish and proceeding as above, using the factor 5 for the calculation.

Recovery experiments are shown in Table 4. It will be seen that the micro-refluxing method used gave results similar to those obtained with Friedmann's method.

(4) B-hydroxybutyric acid.

B-hydroxybutyric acid is determined by difference, the acetone plus acetoacetic acid plus iso-propanol values being subtracted from the "Total Acetone Bodies" value, obtained as follows:

3 ml. of the supernatent solution are placed in the micro-refluxing apparatus with 0.7 ml. acid potassium dichromate solution and a glass bead. The apparatus is closed firmly as before, the water flow started, and the contents boiled for 10 minutes. The solution is taken off the boil and 0.4 ml. 10% potassium dichromate solution run down the cold finger into the solution by means of a syringe. The apparatus is closed firmly again and the solution boiled for a further 10 minutes. The apparatus is then cooled/

TABLE 5.

Recovery of Total Acetone Bodies.

Amount Added. mg./100 ml.	Amount Found. mg./100 ml.	Blank. mg./100 ml.	Error. %
A. Acetone in standard solutions.			
9.50	9.64	0	+ 1.5
9.50	9.32	0	- 1.9
9.50	9.32	0	- 1.9
23.76	23.37	0	- 1.6
23.76	23.37	0	- 1.6
23.76	23.99	0	+ 0.9
B. Acetone in blood.			
9.50	12.97	3.20	+ 2.8
9.50	12.42	3.20	- 2.9
9.50	12.67	2.95	+ 2.3
23.76	27.06	3.20	+ 0.4
23.76	27.68	3.20	+ 3.0
23.76	26.45	2.58	+ 0.4
C. Acetoacetic acid in standard solutions.			
5.76	5.78	0	+ 0.3
11.52	11.56	0	+ 0.3
D. Acetoacetic acid in blood.			
5.76	27.48	21.89	- 3.0
11.52	33.33	21.89	- 0.7

cooled as previously, tipped to mix the contents and 3 ml. transferred to the outer chamber of a Conway dish and the acetone content determined. The factor here is 12.3.

From Table 5 it will be seen that the accuracy of the method for "Total Acetone Bodies" when done on acetone and acetoacetic acid solutions was within ±3%. Estimations of B-hydroxybutyric acid in the quality obtainable (viz., B.D.H. Laboratory Reagent) gave a practically consistent error of 40% in both standard solutions and in blood (Table 6). Various modifications of this method which were tried, such as altering the concentration of chromic acid, varying the time of oxidation and varying the concentration of B-hydroxybutyric acid, all failed to give any increase in the percentage recovery. It was thought that part of this discrepancy might be due to impurity but at our request B.D.H. kindly examined their product which we had used, the sodium salt of B-hydroxybutyric acid, and reported that it gave the expected 78% yield of acetone when examined by Greenberg and Lester's method (1944), and that an approximate determination of purity, by precipitation of the sodium with hydrochloric acid in alcohol, followed by the removal/

TABLE 6.

Recovery of β-hydroxybutyric acid, expressed as acetone.

Amount Added. mg./100 ml.	Amount Found. mg./100 ml.	Blank. mg./100 ml.	Error. %
A. Standard solutions.			
5.08	3.07	0	39.6
5.08	3.07	0	39.6
5.08	3.08	0	39.4
10.15	6.13	0	39.6
10.15	6.13	0	39.6
10.15	6.26	0	38.3
15.24	9.10	0	40.3
15.24	9.10	0	40.3
15.24	9.35	0	38.6
B. Standard solutions in blood.			
5.08	7.38	4.31	39.6
5.08	5.41	2.34	39.6
5.08	5.29	2.34	41.9
10.15	9.84	3.73	39.8
10.15	9.84	3.73	39.8
10.15	10.08	3.73	38.3
15.24	13.16	3.73	38.1
15.24	12.79	3.73	40.5
15.24	13.16	3.73	38.1

removal of the acid into ether, gave a result of approximately 98%. A determination of the sodium content from sulphated ash also gave results equivalent to 98% purity.

From our results it would appear, therefore, that chromic acid only oxidises 60% of the B-hydroxybutyric acid present to acetone under the above experimental conditions, as contrasted with the 78% yield of acetone obtained by Greenberg and Lester (1944) using rather different conditions. As our method of determination yields results consistently 40% low, then to determine the true amount of B-hydroxybutyric acid in a sample the value obtained by subtraction must be multiplied by 5/3. This corrected value added to the previously determined acetone plus acetoacetic acid plus iso-propanol value will then give the true amount of "Total Acetone Bodies" present.

II. Rumen Contents.

The rumen contents are strained through surgical gauze to remove large pieces of food, and the moderately clear liquid is used for the estimations. The protein is precipitated as for blood and the procedure and factors are the same except for iso-propanol, where the factor is 5 and the procedure as follows:

10 ml./

10 ml. of the filtered rumen liquor are placed in a Markham steam distillation apparatus with 2 ml. mercuric sulphate solution and 2 ml. 10% sodium tungstate solution and steam distilled, about 100 ml. distillate being collected. This is repeated with a further 10 ml. liquid and the distillates added.

The combined distillates are washed into a round bottomed flask and 5 ml. mercuric sulphate solution and excess calcium hydroxide added. This solution is distilled direct and the procedure then follows that given for blood.

III. Milk.

As milk contains many reducing substances it is necessary to dilute the solution still further in order to get full oxidation of B-hydroxybutyric acid and iso-propanol to acetone. This is achieved by precipitating the protein as follows:

0.4 ml. milk is added to 4.6 ml. distilled water, 2 ml. barium hydroxide solution and 2 ml. zinc sulphate solution are added. The tube is stoppered, after mixing the contents and centrifuged as before.

The technique employed is the same as for blood except in the case of iso-propanol where the method is similar to that used for rumen contents.

The factors are for free acetone 22.5, for acetone/

TABLE 7.

Recovery of added acetone from milk and urine.

Acetone Added. mg./100 ml.	Acetone Found. mg./100 ml.	Blank. mg./100 ml.	Error. %
A. Milk.			
7.92	7.88	0	- 0.5
7.92	7.88	0	- 0.5
7.92	7.88	0	- 0.5
15.84	16.20	0	+ 2.3
15.84	16.20	0	+ 2.3
B. Urine.			
7.92	7.83	0	- 1.1
7.92	7.83	0	- 1.1
7.92	8.10	0	+ 2.3
15.84	15.93	0	+ 0.5
15.84	15.75	0	- 0.5

acetone plus acetoacetic acid and "Total Acetone Bodies" 30.75 and for iso-propanol 5.

IV. Urine.

The procedure and factors are the same as for blood except in the case of iso-propanol, where the rumen technique is used, and the factor is 5.

In certain acute cases of acetonaemia, the "Total Acetone Body" content of urine rises to several hundred mg./100 ml. In these cases it is advisable to dilute the protein-free liquid still further before oxidation.

Recovery experiments carried out on milk and urine using added acetone gave the results shown in Table 7.

INTERFERENCE.

The following substances were tested for interference in the estimation of acetone bodies, acetic acid, lactic acid, sodium chloride, cholesterol, urea, formaldehyde and acetaldehyde. With one exception, viz., acetaldehyde, the results obtained showed there was no interference. This compound in concentrations as low as 3 mg./100 ml. reacts with the colour reagent to give a slightly opaque orange solution. The interference is lessened slightly after boiling with chromic acid, but not sufficiently to/

to allow of an accurate determination even of total ketones in the presence of such amounts of this material.

RECOVERY FROM MIXED SOLUTIONS.

From the preceding tables it can be seen that using standard solutions of one of the "Acetone Bodies", the errors lay within the range $\pm 5\%$ except in the case of B-hydroxybutyric acid where the percentage yield of acetone was only 60, a discrepancy which could be overcome by the use of an appropriate factor.

Experiments were carried out on standard mixtures of "Acetone Bodies" after complete precipitation and oxidation. It will be noticed that the errors lay within much the same range as before (Table 23).

Estimations were also carried out on standard mixtures in blood and in rumen contents (Table 24) and the experimental errors were again found to lie within the same range. It may be worth mentioning that the concurrent experiments carried out using Van Slyke's method and Denige's reagent gave percentage errors with a much wider range. (Tables 25 and 26).

The method described above gave more consistent results/

results than any of the other methods attempted, and can be carried out easily with very little super: vision. Greenberg and Lester (1944) designed their method for the micro-estimation of keto ne bodies, but owing to the dilutions, which occurred during protein precipitation and subsequent oxidation to acetone, the factors required to determine the acetone in 100 ml. of the original material lay between 20 and 36. Thus any small errors which might occur during the estimation would be exaggerated. Except in the case of milk, where the presence of several reducing agents made concen: tration impractical, this was overcome by using somewhat larger amounts of the material to be analysed, 1 ml. instead of 0.4 ml. The amount of water to be added was decreased from 4.6 to 2 ml., and the protein precipitating agents increased from 2 to 3 ml. each, this being sufficient to precipitate all the protein present in the body fluids.

The chromic acid solution of Greenberg and Lester, 0.6 ml. of a 0.46% solution of potassium dichromate in 15.6 N sulphuric acid, proved, on use, to be inadequate for the oxidation of both B-hydroxy: butyric acid and iso-propanol. Several chromic acid solutions of different strengths were tested, and though the one finally chosen only oxidised 60% of/

of the B-hydroxybutyric acid present, it was found to be adequate in all other respects. A 100% yield of acetone was obtained from standard solutions of iso-propanol, and neither acetone or acetoacetic acid were destroyed by its action.

In Greenberg and Lester's method the following estimations were carried out - acetone, B-hydroxy: butyric acid and total acetone bodies - acetoacetic acid being determined by difference. B-hydroxy: butyric acid was estimated after removing acetone and acetoacetic acid by boiling the protein-free filtrate with a drop of concentrated sulphuric acid for five minutes. As this process has to be carried out with great care, and the exact volume of liquid lost on boiling has to be replaced with distilled water, it was felt that a direct estimation of acetone plus acetoacetic acid would be advantageous. To this end fractions of the protein filtrate, from standard solutions of the two substances, were boiled with varying quantities and strengths of sulphuric acid in a micro-refluxing apparatus, till one was obtained which gave a 100% yield of acetone.

As diffusion appeared to be an easier method of estimation than extraction (Greenberg and Lester), the salicylic aldehyde colour reaction was utilised, and the estimation carried out in a Conway micro-diffusion/

diffusion unit. In order to cover the widest possible range of acetone concentrations, the strongest solution of salicylic aldehyde in potassium hydroxide was employed, 1 ml. in 8 ml. 4 N potassium hydroxide. When weaker solutions of potassium hydroxide and larger quantities of salicylic aldehyde were used the solution precipitated out.

From the results it can be seen that the method is readily applicable to two significant biological materials, in acetonaemia and pregnancy toxaemia, viz., blood and rumen contents. It can equally well be used for milk and urine with no appreciable change in precipitation and oxidation technique.

Although by no means perfect it is, so far as we can ascertain, the first relatively simple method of obtaining a fairly adequate and reasonably accurate differentiation of the various ketone bodies involved in ruminant pathology, and should prove of value in throwing further light on the pathogenesis of various conditions in which acetonaemia is a prominent feature.

SUMMARY.

1. A method has been devised for the estimation of individual acetone bodies and iso-propanol/

iso-propanol within the range 0-120 mg. acetone/100 ml.

2. The basis of the method is the diffusion of acetone into an alkaline solution of salicylic aldehyde with the production of an orange-red colour, the intensity of which is measured in a photo-electric colorimeter.

3. The application of the method to biological materials such as blood, milk, urine and rumen liquor is described, and comparisons are given with other methods.

III. STARVATION KETOSIS.

INTRODUCTION.

Though many workers have produced a starvation ketosis in a variety of experimental subjects, very few appear to have carried out any very detailed investigation into the conditions. The purpose of many of these studies was to elucidate some point of endogenous metabolism that did not involve acetone body formation, and in the majority of the cases the estimation of ketones was confined to total acetone bodies. Without a detailed analysis it is impossible to compare this experimentally produced fasting ketosis with the clinical forms.

Annorexia is a common accompaniment of several pathological conditions which are labelled as "ketosis", such as certain types of definite infections and metabolic disorders of functional origin. It would be a definite advantage, to be able to judge if the ketosis, noted in these conditions, was solely due to the loss of appetite, or was caused or aggravated by the primary disturbance. Further, there is a theory of long standing that post parturient dyspepsia of cattle, (or bovine ketosis as it is often called), and pregnancy toxaemia of ewes are caused by an inadequate caloric intake. (Sampson and Hayden. 1936). It was felt, therefore, that it would be of interest to make/

make a detailed study of starvation ketosis in these animals, especially as earlier experiments on the cow appear to have yielded rather conflicting results. Thus, in 1923, Sjollema and Van der Zande carried out one of the earliest experiments on starvation ketosis by fasting milch cows for two days, following an injection of phlorizin. They found that only a slight ketonuria was produced and came to the conclusion that the cow does not readily produce much acetone, unless in certain diseased conditions. Later, Carpenter (1929) fasted two steers for five and ten days respectively, and found no increase in the urinary acetone bodies. He concluded that the ruminant was exceptionally resistant to fasting ketosis, a belief which lasted for about fifteen years.

In 1943 Forbes produced an experimental fasting ketosis in ruminants by giving them half, or less, of their nutritional requirements for about ten days. He took two cows at peak lactation and fed half the Morrison minimum requirements for maintenance and milk production, in one case for twenty-one days and in the other for nine. In both animals he found a rise in total blood ketones. A third cow was fed 6 lbs. of timothy hay daily for ten days and again a rise in total blood ketones was noted.

Forbes/

Forbes also used two cows later in lactation, viz., at 8 weeks and 14 weeks following parturition. After feeding the former a restricted diet and the latter only timothy hay for ten days, he found there was no response. He also kept a dry cow, either non-pregnant or in very early pregnancy, on a maintenance diet for several days and produced no response, while cows in late pregnancy on the same diet showed a rise in total blood ketones.

Both Carlström (1950) and Holmes (1950) starved cows in the post-parturient period and found a marked urinary excretion of acetone bodies. Holmes believed that the sudden fall in milk yield, which he noted on starvation lessened, to a certain extent, the severity of the ketonuria produced.

Even before Hopkirk (1934) produced a ketosis on under-feeding fat pregnant ewes, the general opinion was that pregnancy toxaemia developed under conditions of poor feeding, more particularly in the ewe carrying more than one lamb. Greig (1929) considered that a sudden change in grazing was a predisposing factor, and the Onderstepoort workers (Groenewald et al. 1941) went further and suggested that any sudden change in environment or handling might cause a pregnant ewe to feed poorly. The same workers (Clark et al. 1943) underfed and starved/

starved ewes in late pregnancy, and produced a starvation ketosis, the clinical symptons of pregnancy toxaemia being manifested in some cases. They also noted that several of the ewes refused food altogether after a starvation period of from five to thirty days. Under very severe conditions, over a much longer period of time, viz., one to three months on low rations, they found a ketosis could be produced in the non-pregnant ewe. Sampson and Boley (1940) underfed and starved ewes in pregnancy and though they produced a ketosis in their sheep, none of the clinical signs of pregnancy toxaemia were observed. They suggested that as the disease developed over several weeks of underfeeding, the effects produced would be more severe than those observed over a short period of experimental fast.

In view of the fact that no detailed biochemical study of starvation ketosis had been made, a number of cows and ewes were starved completely for several days. The cows chosen were for the most part at peak lactation, about three weeks after calving, when the tendency to develop bovine ketosis is greatest. Likewise one non-pregnant ewe, and two in late pregnancy, the time at which pregnancy toxaemia usually develops, were taken for these experiments/

experiments. Each of the individual acetone bodies was followed, viz., acetone, acetoacetic acid, B-hydroxytubyric acid and iso-propanol.

STARVATION KETOSIS IN THE DAIRY COW.

Method.

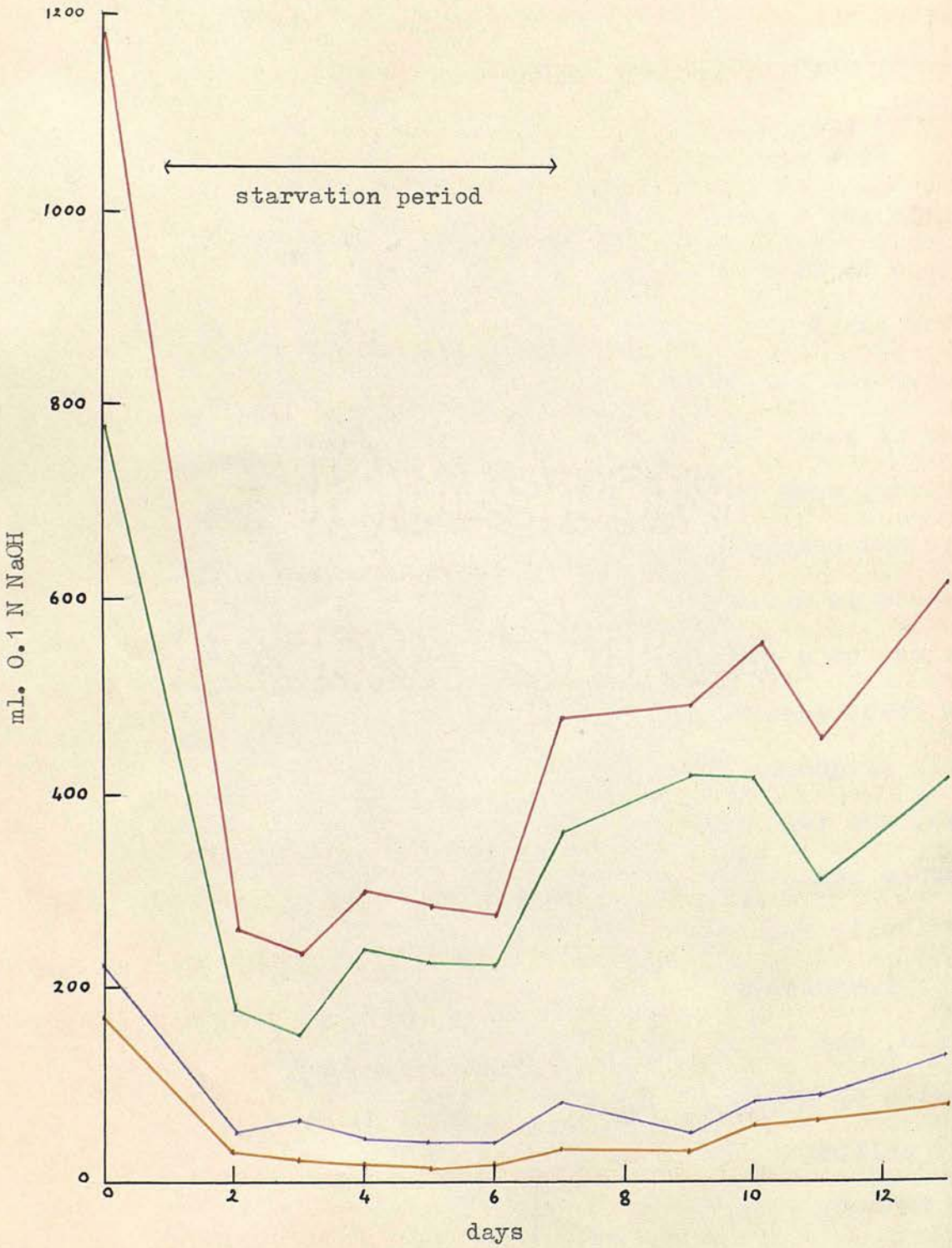
Five cows at peak lactation were kept in their stalls for a period of from five to six days without access to food or bedding, but with an adequate water supply. The feeding before and after the starvation period was the normal feeding for the time of year, though in winter, on resumption of feeding, only hay and roots were given for a few days and concentrates added later. In addition one cow in mid-lactation (13 weeks after parturition) was put on a maintenance diet for a week, one dry cow about a month from calving and one dry cow in early pregnancy were starved completely for five days, and two cows with sub-clinical ketosis were starved completely for a few days, one having previously been taken off concentrates for seven days.

Immediately prior to and during the starvation period, and for a short time afterwards, daily samples of blood, rumen contents, milk and urine were collected, the blood usually being taken from the mammary vein, and the rumen contents by stomach/

FIGURE 2.

The effect of starvation on the volatile fatty acids in the rumen.

Total volatile fatty acids —, acetic acid —, propionic acid —, butyric acid —.



stomach tube. Two of the cows used had self-sealing rumen fistulae, but owing to the thick consistency of the rumen contents and the narrowness of the catheter employed, sampling proved a rather slow and tedious process. In some cases 24 hour samples of urine were collected. The daily milk yields of each cow were noted.

Blood sugar determinations were carried out using the method of Hagedorn and Jensen (1929). Rumen volatile fatty acids were estimated by McAnally's modification (1944) of Friedmann's steam distillation method and Elsden's chromatographic technique (1946). The acetone bodies were determined by the method previously described. In some cases the pH of the rumen liquor was determined electrometrically.

Results.

Volatile acids.

The total volatile fatty acid content of the rumen samples fell sharply on starvation in all cows, followed by a rise after re-feeding (Fig. 2). Each of the three individual acids, acetic, propionic and butyric, fell by amounts roughly proportional to their initial concentrations. These changes in concentration with fasting were statistically significant at 0.1% level. The unexpected increase in total/

TABLE 8.

pH of rumen contents.

Cow.	Days of Fast.					
	0	1	2	3	4	5
3.....	6.4	8.0	8.6	8.2	7.7	7.8
37.....	7.3		8.9	7.6	7.2	6.7
27.....	6.6	8.5	7.7	7.8	7.7	7.7
Mean.....	6.76	8.25	8.40	7.86	7.53	7.40

total volatile fatty acids noted on the third day of fast, after the minimum value reached on the second day, may also be regarded as statistically significant. The trend in total volatile fatty acids after re-feeding was also somewhat unexpected, in so far as the initial increase apparently received a setback, the levels on the fifth day of re-feeding being slightly lower than those on the fourth and seventh days.

pH of the rumen contents.

Associated with the change in total volatile fatty acids of the rumen contents, were the changes in pH shown in Table 8. The pH of the rumen contents rose on starving from an average value of 6.7 at the outset, to a maximum value of 8.4 on the second day of starvation, followed by a fall to 7.4 on the fifth day. After re-feeding commenced, the average pH value fluctuated apprec: iably from day to day within the range 6.5 - 7.5.

Milk Yield.

All cows showed a rapid and significant fall in milk yield on fasting (Table 9). The rate of fall of milk yield of the cows at peak lactation was very marked at first, but the curve tended to flatten out towards the fifth day, when the average yield of milk was 12 lbs. On re-feeding the recovery/

TABLE 9.

Milk yield (in lbs. per day).

At peak lactation.

Cow.	Days of Fasting.						Days of Re-feeding.						
	0	1	2	3	4	5	1	2	3	4	5	6	7
3.....	47.5	23.0	17.5	13.0	14.0	11.0	13.0	15.0	15.0	15.5	16.5	20.5	23.5
10.....	33.5	22.0	17.0	12.0	9.0	14.0	14.0	12.5	16.0	18.5	21.5	23.5	24.5
13.....	60.5	30.5	17.0	16.0	13.0	13.5	8.5	11.0	13.5	16.0	17.0	21.5	20.5
20.....	78.0	38.0	26.0	25.5	19.0	20.0	18.0	40.5	43.5	51.0	55.0	54.5	55.5
37.....	45.0	33.5	14.0	13.0	8.5	7.0	5.0	4.5	7.0	6.0	11.0	7.0	6.0
Mean.....	52.9	29.4	18.3	15.9	12.7	13.1	11.7	16.7	18.0	21.4	24.2	25.4	26.0

TABLE 9 (Contd).

Milk yield (in lbs. per day).

13 weeks after parturition.

Cow.	Days of Mairtenance Diet.							Days of Full Rations.					
	0	1	2	3	4	5	6	1	2	3	4	5	6
32.....	36.5	30.5	24.0	22.5	22.5	23.5	22.0	26.5	28.0	30.0	32.5	35.0	34.0

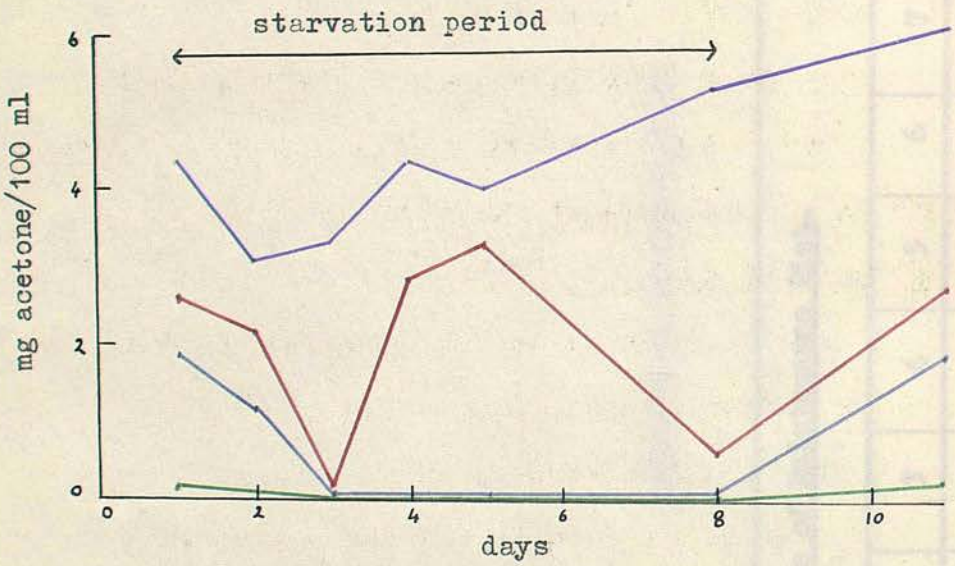
Subclinical Bovine Ketosis.

Cow.	Days of Maintenance Diet.								Days of Fast.			Days of Full Rations.	
	0	1	2	3	4	5	6	7	1	2	3	1	2
11.....	45.0	42.5	33.0	34.0	29.0	29.5	29.0	29.5	22.5	12.0	12.5	23.5	28.0

FIGURE 3.

The effect of a maintenance diet on the ketones of a cow at mid-lactation (β-hydroxy butyric acid was the only acetone body present).

Blood——, rumen contents——, milk——, urine——.



recovery in milk yield over the period studied was slow, especially with cow No. 37 which showed marked loss of condition during the fasting period.

Semi-starvation of a cow 13 weeks after parturition, and one with sub-clinical ketosis, caused a drop in milk yield over the period of fast. The rate of fall of milk yield was much less marked than that described above, and the average milk yield reached on the fourth day of maintenance diet was 25.5 lbs., compared to 40.5 lbs. in the prefast period. After seven days on a maintenance diet the cow with subclinical ketosis was starved completely for three days, when the milk yield fell still further to an average value of 12 lbs.

Acetone Bodies.

Removal of concentrates from the diet of a cow at mid-lactation or a cow in early pregnancy caused no rise in blood ketones (Fig. 3). During the whole of the experiment only B-hydroxybutyric acid was present in the blood, rumen contents, milk and urine, and although the amount present varied from day to day it was well within the normal range, viz., below 10 mg./100 ml. in the blood.

Complete starvation of a dry cow, about a month before calving, caused a marked rise in blood ketones (Table 27). Starvation was commenced in the afternoon/

TABLE 10.

Acetone Bodies during fasting a dry cow in late pregnancy.

(As percentage of total)

Blood.	Days of Fast.					
	0	1	2	3	4	5
Acetone.	0	0	4.06	6.38	14.03	27.73
Acetoacetic acid.	0	0	0.71	3.38	9.32	3.92
B-hydroxy butyric acid	100	100	95.23	89.66	76.04	67.07
Iso-propanol.	0	0	0	0.58	0.61	1.28

in the afternoon and in the twelve hours between the first two samples no change was noted, but subsequent samples showed a progressive increase in all the acetone bodies. The greatest rise was encountered in the B-hydroxybutyric acid fraction, though the percentage content fell (Table 10). Free acetone rose from an almost negligible amount on the second day to about 27% of the total on the fifth day of fast, a marked rise occurring on the fourth and fifth days. Acetoacetic acid showed a more gradual rise than acetone attaining a maximum value of 9% of the total acetone bodies on the fourth day, with a subsequent fall to 4% on the fifth. The rise in iso-propanol concentration was very slight; the maximum concentration reached was some 1.2% of the total acetone bodies on the fifth day of fast.

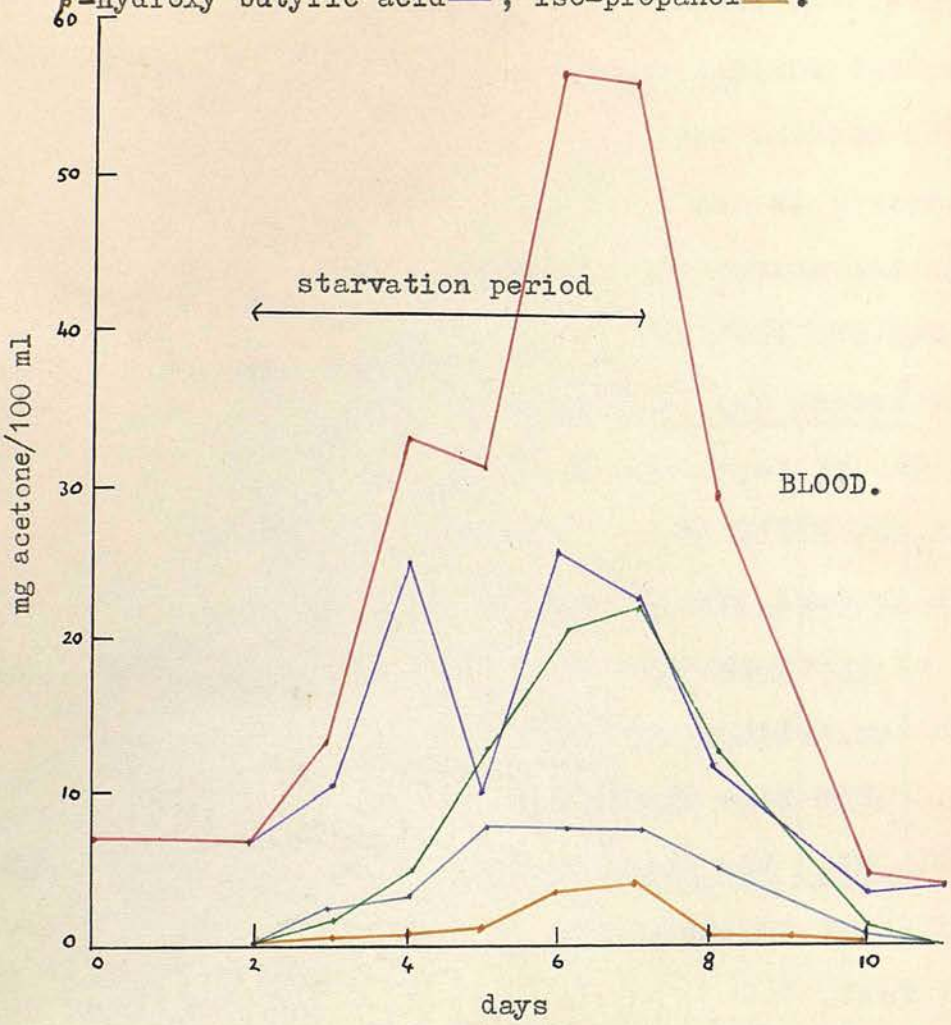
The five cows starved at peak lactation all showed a degree of ketonaemia, as shown in Table 28 which gives the average figures for five animals, and Figs. 4 and 5 which show the variations in individual constituents in a representative animal (Cow No. 10). This ketonaemia was associated with loss of condition and a fall in milk yield without any other sign of illness or loss of appetite.

As will be noted, the rise in total acetone bodies/

FIGURE 4.

The effect of starvation on the blood and rumen ketones of a cow at peak lactation.

Total acetone bodies —, acetone —, acetoacetic acid —, β-hydroxy butyric acid —, iso-propanol —.



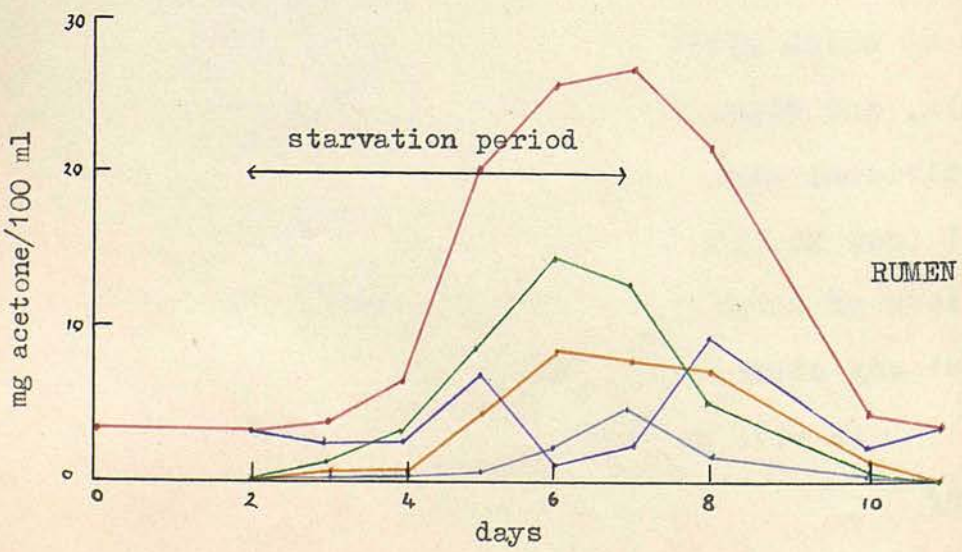
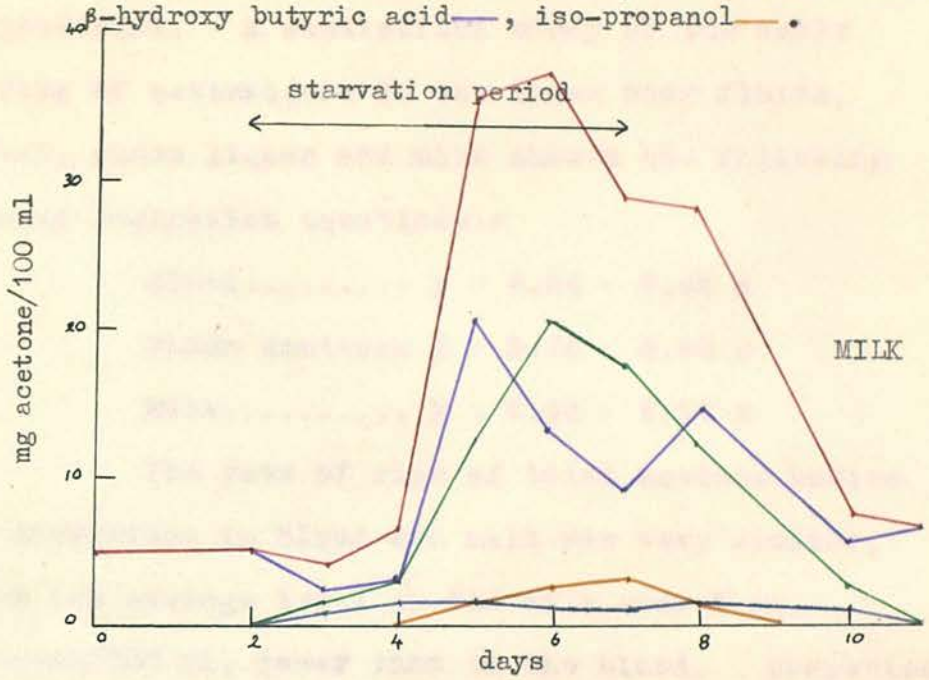
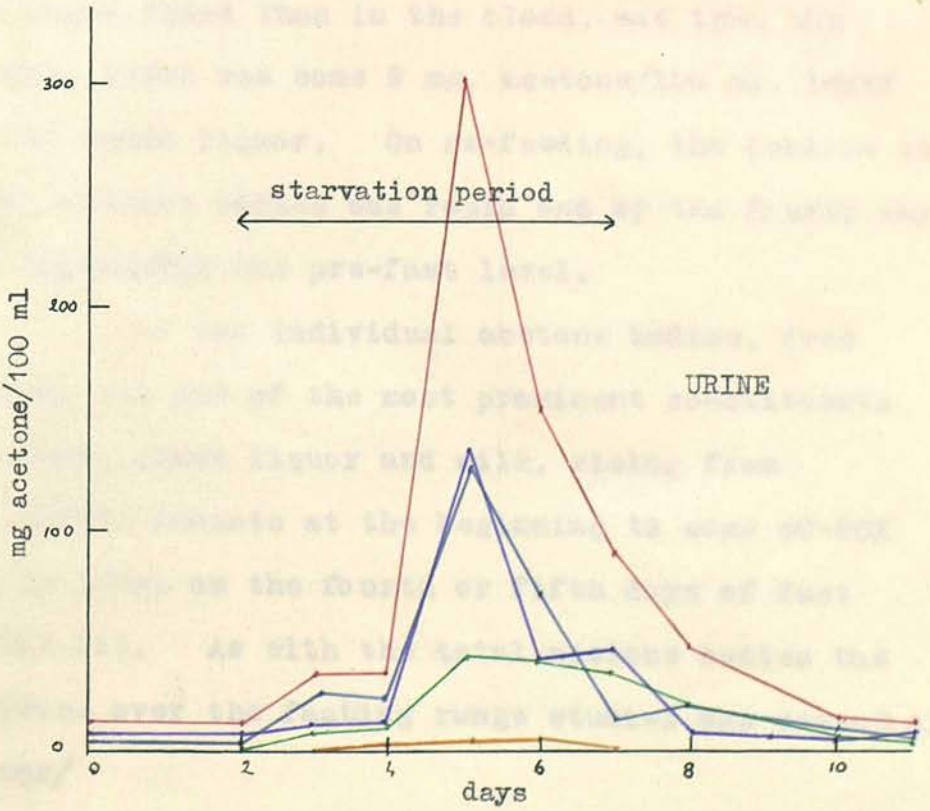


FIGURE 5.

The effect of starvation on the milk and urine ketones of a cow at peak lactation.

Total acetone bodies —, acetone —, acetoacetic acid —, β-hydroxy butyric acid —, iso-propanol —.





bodies on starvation was progressive and highly significant. A statistical study of the whole series of estimations on the three body fluids, blood, rumen liquor and milk showed the following linear regression equations:-

Blood..... $y = 6.34 + 7.48 x$

Rumen contents $y = 3.45 + 4.22 x$

Milk..... $y = 5.42 + 5.14 x$

The rate of rise of total acetone bodies on starvation in blood and milk was very similar, with the average level in the milk some 5 mg. acetone/100 ml. lower than in the blood. Comparison for blood and rumen contents showed that the total acetone bodies on starvation rose less sharply in the rumen fluid than in the blood, and that the average level was some 9 mg. acetone/100 ml. lower in the rumen liquor. On re-feeding, the decline in total acetone bodies was rapid and by the fourth day had approached the pre-fast level.

Of the individual acetone bodies, free acetone was one of the most prominent constituents in blood, rumen liquor and milk, rising from negligible amounts at the beginning to some 40-50% of the total on the fourth or fifth days of fast (Table 11). As with the total acetone bodies the increase over the fasting range studied was essentially linear/

TABLE 11.

Acetone bodies during fasting.

(As percentage of total)

	Days of Fast.					
	0	1	2	3	4	5
Blood:						
Acetone.....	0.45	12.38	19.95	28.12	41.04	33.58
Acetoacetic acid.....	9.82	13.63	13.60	14.50	16.13	9.79
B-hydroxybutyric acid..	89.73	73.76	65.02	52.49	37.85	52.35
Iso-propanol.....	0.00	0.25	1.43	4.89	4.98	4.28
Rumen:						
Acetone.....	0.00	12.32	25.46	30.41	42.97	50.86
Acetoacetic acid.....	5.63	3.78	13.09	4.56	5.50	7.32
B-hydroxybutyric acid..	94.37	77.32	50.32	46.75	28.36	17.56
Iso-propanol.....	0.00	6.58	11.13	18.28	23.17	24.26
Milk:						
Acetone.....	0.00	19.69	20.92	32.64	55.22	59.05
Acetoacetic acid.....	0.00	3.82	15.51	3.15	6.82	5.71
B-hydroxybutyric acid..	100.00	75.78	62.70	60.08	32.48	28.80
Iso-propanol.....	0.00	0.71	0.87	4.13	5.48	6.44
Urine:						
Acetone.....	5.91	1.04	1.81	9.46	24.03	26.25
Acetoacetic acid.....	19.72	53.44	63.04	47.40	39.24	44.50
B-hydroxybutyric acid..	74.37	45.26	34.16	36.01	33.95	23.72
Iso-propanol.....	0.00	0.26	0.99	7.13	2.78	5.53

linear, and equal to 3.39, 2.83 and 3.60 mg. acetone/ 100 ml./day for blood, rumen contents and milk respectively.

Acetoacetic acid showed a more gradual rise than acetone in the blood, attaining a value of 16% of the total acetone bodies on the fourth day, with a subsequent fall to 10% on the fifth. The levels reached in the milk and rumen contents, about 6% of the total acetone bodies, were maintained with minor fluctuations throughout the experiment except for a sudden rise to about 14% of the total acetone bodies on the second day of fast. As with the total acetone bodies and free acetone, the increase over the fasting range studied was essentially linear, and equal to 0.70, 0.51 and 0.30 mg. acetone/100 ml./day for blood, rumen liquor and milk respectively.

B-hydroxybutyric acid showed a rise in the blood over the period of fasting much the same as that of free acetone, though the percentage content fell to 38% of the total on the fourth day of fast compared to 90% of the total acetone bodies in the pre-fast state. The levels in the milk and rumen liquor behaved, with slight variations, very much the same during the fasting period, attaining a point of maximum concentration of 16 and 8 mg./100 ml. respectively/

respectively on the third day of fast. The per: centage in the rumen liquor showed a much greater fall than in the blood and milk, attaining a value of about 17% of the total acetone bodies on the fifth day of fast, compared to 28% in the milk. The increase over the fasting range studied was essentially linear, and equal to 2.85, 0.22 and 0.85 mg. acetone/100 ml./day for blood, rumen con: tents and milk respectively.

Iso-propanol showed a more gradual rise than free acetone in the rumen liquor, attaining a value of 24% of the total acetone bodies on the fifth day of fast. The levels reached in the blood and milk were much lower, viz., 5% and 6% of the total acetone bodies, the increase over the range studied being essentially linear, and equal to 0.55, 1.09 and 0.40 mg. acetone/100 ml./day for blood, rumen contents and milk respectively.

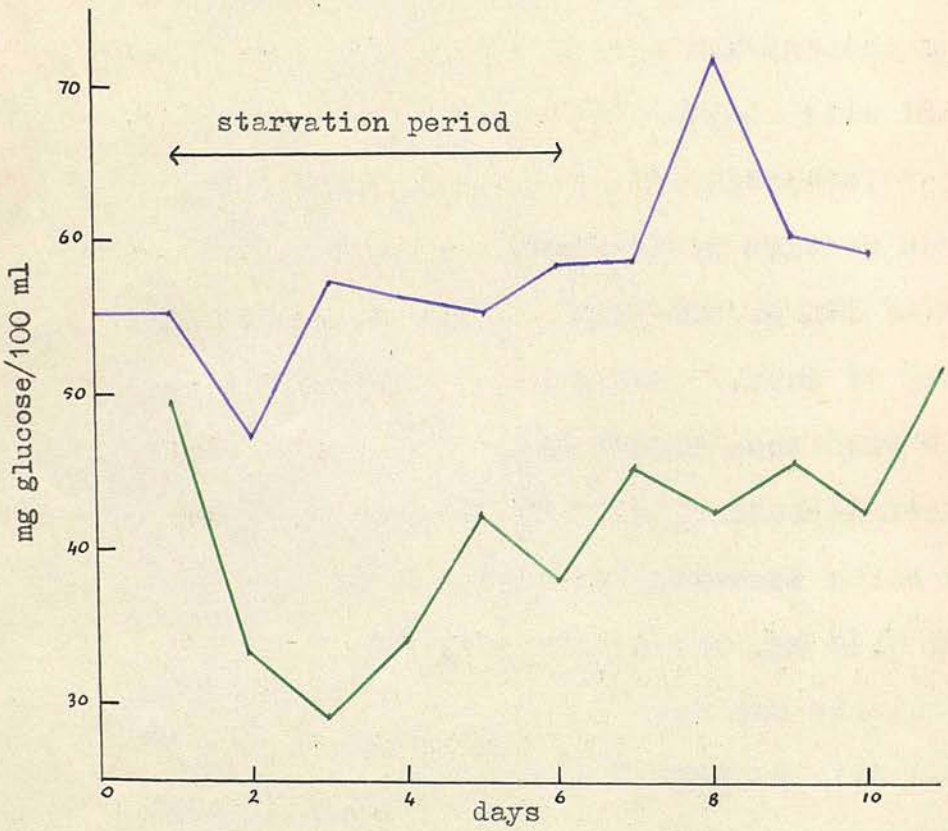
All the cows studied showed an increase in the total acetone bodies excreted in the urine on fasting, the most prominent fraction being usually acetoacetate which, at times, reached concentrations of more than 50% of the total acetone bodies present. B-hydroxybutyric acid, acetone and iso-propanol were also excreted, but in decreasing proportions in that order. Normally B-hydroxybutyric acid only is excreted.

Blood/

FIGURE 6.

The effect of starvation on the blood sugar levels.

average of five cows at peak lactation——,
a cow with sub-clinical ketosis——.



Blood Sugar.

The cows starved at peak lactation all showed a statistically insignificant fall in blood sugar on the first day of starvation (Fig. 6) with a subsequent rise to the normal value on the second or third days. There was a slight temporary rise on re-feeding. Only two of the cows showed a fall in the blood sugar value to below the normal range. In cow 37 which lost condition markedly and whose milk yield remained low, the blood sugar value continued to rise on re-feeding, reaching a value above the normal range before returning to the pre-fast value. One cow gave abnormally high values on the first day of fasting, and on re-feeding, probably associated with the fact that she was in a very excited state on these occasions.

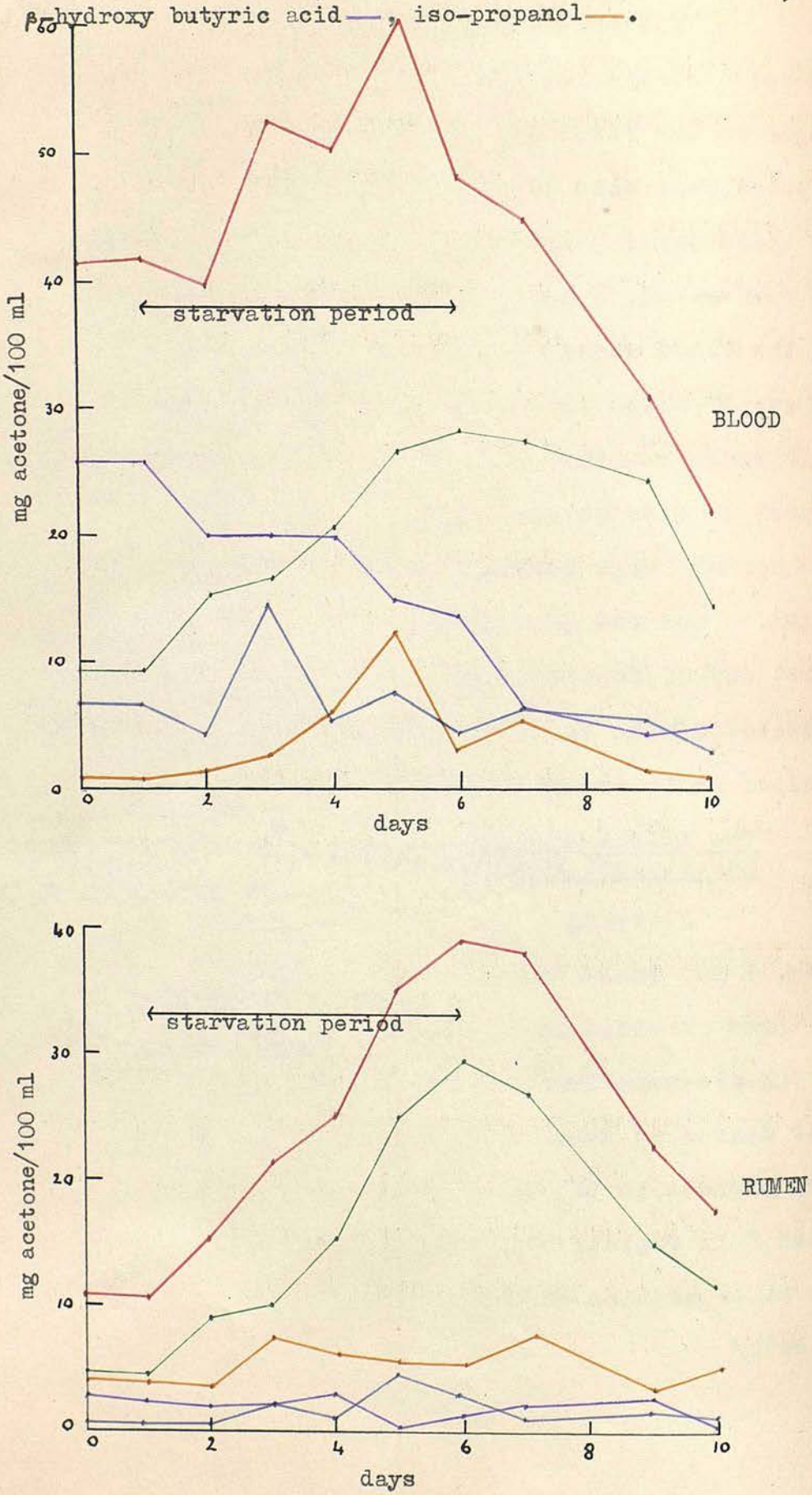
The Effect of Starvation on Cows with Subclinical Ketosis.

Starving a cow with subclinical ketosis (Fig. 7 and Table 29) gave results somewhat similar to those obtained on starving cows at peak lactation. In the pre-experimental period cow No. 27 showed a fair degree of ketosis though the predominant acetone body present in the blood was B-hydroxybutyric acid which fell rapidly on starvation. The response of the other acetone bodies and the total acetone bodies in both/

FIGURE 7.

The effect of starvation on the blood and rumen ketone levels of a cow with sub-clinical ketosis (no. 27).

Total acetone bodies —, acetone —, acetoacetic acid —, β-hydroxy butyric acid —, iso-propanol —.



in both the blood and rumen was much the same as above except for iso-propanol in the rumen contents. The relative proportions of this fraction fell though the concentration remained more or less steady. In another cow in which the ketosis in the pre-fast period was not so severe, all the acetone bodies in the blood, rumen liquor and milk responded in the same manner on starvation. In these animals acetoacetate and B-hydroxybutyric acid were again the main excretory products in the urine.

Blood sugar was determined in only one of the sub-clinical ketosis cases. The value lay just within the normal range in the pre-experimental phase and fell on starving to about 30 mg./100 ml. It rose again gradually before re-feeding and reached the normal value of about 50 mg./100 ml. a short time later (Fig. 6).

STARVATION KETOSIS IN THE EWE.

Method.

Two ewes in the last fortnight of pregnancy and one non-pregnant ewe were removed from the herd and kept in single pens for four days without access to food or bedding, but with an adequate water supply. As the sheep did not have rumen fistulae only blood samples/

TABLE 12.

Acetone bodies during fasting.

(As percentage of total).

Blood.	Days of Fast.				
	0	1	2	3	4
A. Non-pregnant ewe.					
Acetone.....	0	0	0	0	3.64
Acetoacetic acid.....	0	0	0	10.14	4.09
B-hydroxybutyric acid.	100	100	100	89.86	91.10
Iso-propanol.....	0	0	0	0	1.17
B. Pregnant ewe.					
Acetone.....	27.18	20.73	17.31	26.50	16.47
Acetoacetic acid.....	18.22	46.96	16.35	16.58	13.98
B-hydroxybutyric acid.	53.93	29.49	64.96	56.34	68.82
Iso-propanol.....	0.67	2.82	1.38	0.58	0.73
C. Pregnant ewe.					
Acetone.....	4.61	3.23*	3.77		
Acetoacetic acid.....	0.81	6.10	10.81		
B-hydroxybutyric acid.	94.58	90.67	85.42		
Iso-propanol.....	0	0	0		

* Ewe lambed between 1st and 2nd days of fast.

samples (from a jugular vein) were taken daily over the experimental period.

The acetone bodies were determined as previously described.

Results.

The total acetone bodies rose in the blood of the non-pregnant ewe on starvation, but it was not until the third and fourth days of starvation that any acetone body other than B-hydroxybutyric acid, was found, (Tables 12 and 30). On the third day a small percentage of acetoacetic acid was found, and on the fourth day acetone and iso-propanol were added.

Though pregnant ewe B. appeared normal in all respects she was found, at the commencement of the experiment, to have a subclinical ketosis, all four acetone bodies being present in the blood in the prefast period. The rise in total acetone bodies on starvation was progressive, and brought about by a rise in all the constituent ketones. The greatest rise was encountered in the B-hydroxybutyric acid fraction, and the least in the iso-propanol fraction. After the first day of fast a very high percentage of acetoacetic acid was found in the blood. Ewe B. lambed normally two days after re-feeding.

Pregnant ewe C. also showed a degree of ketonaemia in the/

in the pre-fast period, though much less severe than that found in ewe B. Starvation produced a rise in total acetone bodies, as in the previous case, with a rise in acetoacetic acid after the first day of fast. Ewe C. lambed after two days of fast and the blood sample taken immediately after showed a drop in total acetone bodies.

None of the sheep showed a loss of appetite after the starvation period.

DISCUSSION.

Owing to the loss of carbon dioxide to the air on removal from the rumen, the pH values estimated for the rumen liquor would be higher than the actual values in the rumen itself, but it is evident nevertheless that a rise in pH does occur on starving, coinciding, as would be expected, with the fall in volatile fatty acids. This fall is itself fairly easily understood, as bacterial action in the rumen is bound to slow down with the withholding of fresh metabolites. This was confirmed by concurrent bacteriological studies to be reported elsewhere. The fact that all three acids, acetic, butyric and propionic, fall in about the same proportion suggests that their formation reactions are equally affected. The slight rise encountered towards the end of the starvation/

starvation period may be associated with a change in the volume of the rumen contents.

Three cows, one 8 weeks after parturition (Forbes 1943), one 13 weeks after parturition (above), and one in the dry state, either non-pregnant or in very early pregnancy, (Forbes 1943 and above), were kept on maintenance diets for from five to seven days without showing any increase in the blood ketones. In addition Forbes (1943) put a fat cow, 14 weeks after parturition, on what was virtually a ten day fast without any response. This particular cow was only given enough hay to keep her quiet while the calf was receiving grain. From this it would seem that the non-pregnant cow, from 8 weeks after parturition to the dry state, has sufficient glycogen stores in her body to carry on, during a five to seven day period of semi-starvation, without resorting to excessive fat catabolism, and, further, that she can resist more or less complete starvation for ten days from 14 weeks after parturition. In late pregnancy and at peak lactation, however, the cow's glycogen stores are apparently inadequate and on starvation she is forced to live on her own fat. As it is an established fact that when fat is metabolised acetoacetate is formed (Weinhouse et al. 1944, 1945), it is not surprising to find/

to find, in the cows at peak lactation, that this is one of the first acetone bodies to appear in quantity, more rapidly in the blood than in the rumen and milk, so that presumably it reaches these latter by diffusion from the blood stream. In the dry pregnant cow, however, the rise in B-hydroxybutyric acid and acetone were more marked than that of acetoacetic acid, and during the period of fast the concentrations of B-hydroxybutyric acid were very much higher than those encountered during the starvation of a cow at peak lactation. It has been stated by many workers that acetoacetate is toxic, and certainly the blood levels appeared to be kept low in these experiments with free acetone appearing in large quantities. The cow, therefore, is proved to be quite capable of producing a considerable degree of acetonaemia even in the absence of disease.

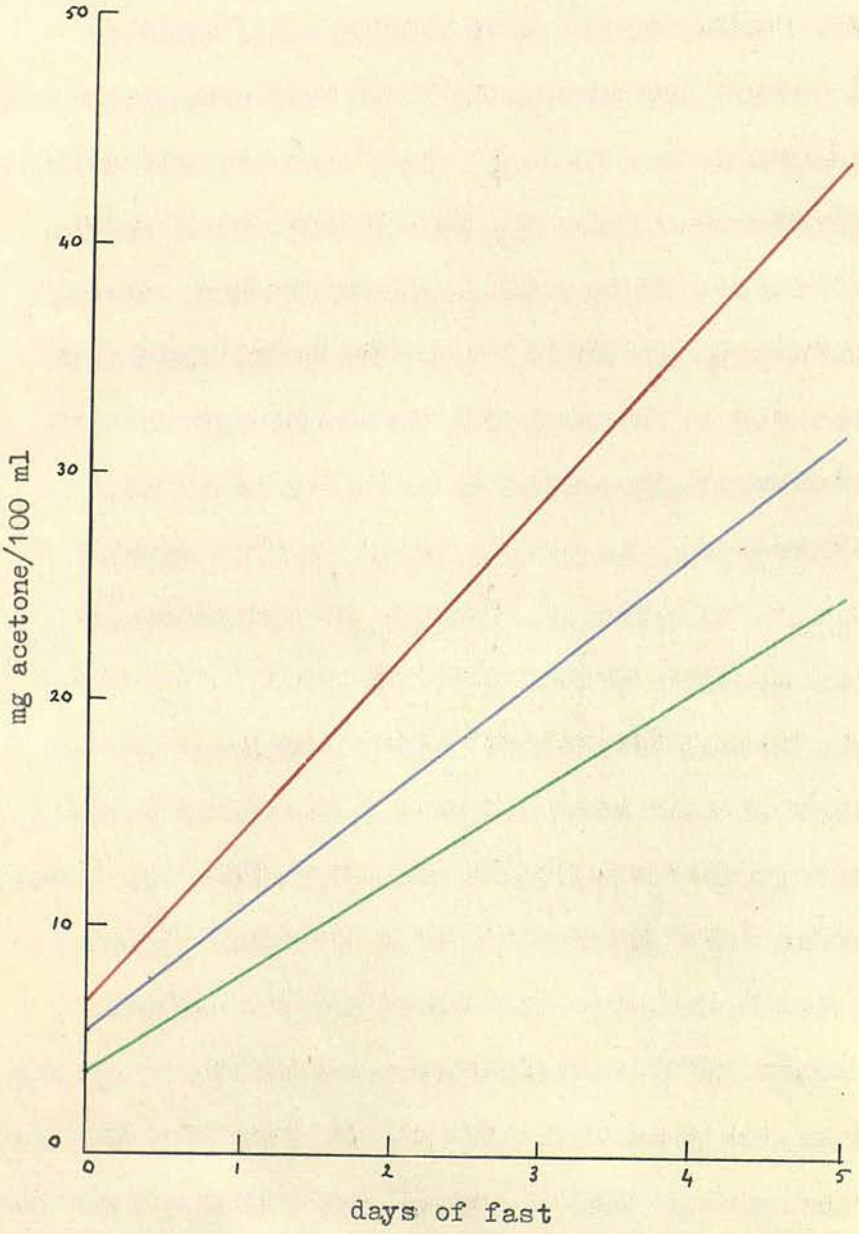
Shaw, Powell and White, (1942), explain the predominance of acetoacetate in the urine in ketosis by a change in the renal threshold for this substance. The fact that this predominance also occurs on fasting, with the knowledge that acetoacetate is not normally found in the blood stream or urine, suggests that this excess excretion may not be due to a change in renal threshold, but is merely the natural reaction of the kidneys to the presence of a substance with a low renal threshold.

If we take/

FIGURE 8.

The regression of concentration of total acetone bodies on days of fast for blood, milk and rumen contents.

Blood——, rumen contents——, milk——.



If we take as a base line the relative proportions of the acetone bodies in the blood, we can assess the changes which occur in the rumen and milk. The linear regression of concentration of total acetone bodies on days of fast for the three, blood, milk, and rumen contents (Fig. 8), shows that the three lines are very similar, though the levels vary, being lower in the milk and rumen contents than in the blood. The relative proportions of acetone and iso-propanol in the blood and milk are very similar, though slightly higher in the latter (Table II). From the point of view of the blood, therefore, the mammary gland appears to act as an excretory organ ridding the body of some of the excess acetone bodies. There is very little change in either blood or milk in the proportions of acetoacetate over the fasting period, and the proportion of B-hydroxybutyric acid falls by much the same amounts in both fluids.

The evidence suggests a direct diffusion from the blood into the milk with very little, if any, utilisation of the acetone bodies by the mammary gland, except perhaps in the case of acetoacetate and B-hydroxybutyric acid towards the end of the fast. Shaw (1942) has shown that the mammary gland does not utilise acetoacetate but can use B-hydroxybutyric acid for energy purposes.

The rise/

The rise in total acetone bodies in the rumen is very much slower than in the blood, but the relative proportions of acetone are very similar, as both show an increase on fasting to about 50% of the total acetone bodies. In both there is very little change in the proportions of acetoacetate over the fasting period. The rise in the proportion of iso-propanol is very much more marked in the rumen than in the blood, as is the fall in B-hydroxy: butyrate.

The acetone bodies probably reach the rumen by way of the saliva and possibly also by direct diffusion. In no case does iso-propanol appear in the blood stream before it appears in the rumen contents. This suggests that the site of formation is that organ, where there is the greatest increase in concentration. If iso-propanol is formed from one of the other acetone bodies it would appear that, directly or indirectly, B-hydroxybutyric acid might be the precursor, as this is the only fraction to decrease in proportion to the total acetone bodies. It must be remembered, however, that as this con: stituent comprises the bulk of the acetone bodies present in the pre-fasting stage, a rise in the con: centration of any of the others is bound to cause a fall in the relative proportion of this fraction. It should/

It should be noted, too, that in the rumen contents the average values for iso-propanol remain about half those of free acetone, taken at the same time, over the period covered. This suggests a close relationship between acetone and iso-propanol in the rumen, which is in keeping with their chemical composition and it would appear therefore that acetone might well be a precursor of iso-propanol in the rumen. It is of course possible that the precursor is some other substance not estimated in these experiments.

Forbes in 1943 found that on putting goats in late pregnancy and cows at peak lactation on half rations for ten days, the blood sugar fell steadily from about 50 to 30 mg./100 ml. only rising again when full rations were given. Hodgson, Riddel and Hughes (1932) found the blood sugar in dairy cattle fasted for nine days fell gradually during that period, and on the seventh day of fasting started to rise again, only reaching normal a few days after re-feeding. During the complete starvation described in the preceding experiments, it was found that though the blood sugar fell at the beginning of the experiment the drop was not significant, that after two or three days it rose again to the normal level, and there was little change observed on re-feeding.

The difference/

The difference may lie in the milk production. On starvation the milk yields of all the cows used in our experiments fell rapidly by amounts ranging from 20 to 60 lbs. daily, the greatest fall being observed in the highest yielding cow. Forbes does not mention the milk yields of the cows he fed half ration. It may be that he noted no sudden change, or that he was not interested in the yield. Shaw, (1946), recorded a sudden drop in milk yield on fasting milking cows but carried out no sugar estimations at that time. Hodgson et al. used heifers of 1½ to 2 years old so milk production did not enter into their experiments.

A sudden drop in milk yield in a high-yielding cow would presumably slow down the passage of metabolites from the blood stream to the mammary gland, with a resultant conservation of body resources which should help in the maintenance of normal blood levels. If the milk yield of a cow on half rations did not fall appreciably, a very great strain would be put on the animal's resources with a resultant fall in blood sugar. In the preceding experiments the starvation was sudden, and so the body took a day or two to regain its internal balance, during which there was a temporary fall in blood sugar. It would appear that the blood sugar of an adult milking/

milking cow can, as in other species such as man and the dog, find its own level again on starvation, there being at the beginning a temporary upset which the body mechanisms can soon control.

Results obtained by starving a case of sub-clinical ketosis were in the main very similar to those obtained on starving a normal cow at peak lactation. All the acetone bodies, except iso-propanol in the rumen, rose in much the same way over the fasting period. The relative proportions of iso-propanol in the rumen fell though the con: centration remained much the same. It will be noted that the concentration of iso-propanol in the rumen of a cow with sub-clinical ketosis is very much the same as that found in a normal lactating cow after a five day fast, and so it would appear that either the maximum amount of iso-propanol is being produced under these circumstances or the rate of production is being balanced by the rate of metabolism by the rumen bacteria.

The blood sugar did not return to the normal value after the initial drop till some time after re-feeding. This may have been due to a long period of subclinical ketosis having an adverse effect on the general health and body stores of the animal. The controlling mechanisms of the body would thus take/

take longer to regain the equilibrium point.

About a week after re-feeding both the cows previously suffering from subclinical ketosis were entirely normal.

As in the cow at peak lactation and the dry cow in late pregnancy, complete starvation produced a degree of ketosis in the ewe in late pregnancy. A similar rise was encountered in the two species in total acetone bodies, acetone and B-hydroxybutyric acid, though the rise in acetone was less, and in B-hydroxybutyric acid greater, in the sheep than in the cow at peak lactation. These results were very similar to those obtained on fasting a cow in late pregnancy. The rise in acetoacetic acid was rather more pronounced in the sheep, after the first day of fast, than in the cow at the same time, and, over the period of fast studied, the rise in iso-propanol was very slight in both the sheep and the cow in late pregnancy.

If, as has been suggested previously, iso-propanol is formed from acetone in the rumen, the lower percentages of both acetone and iso-propanol in the ewe and cow in late pregnancy, would tend to confirm this, as well as indicating that in late pregnancy neither the cow nor the sheep produce acetone in the same quantities as the cow at peak lactation.

If/

If the ketosis observed on starvation was due, primarily, to the excessive catabolism of body fat, with the production of abnormal quantities of acetoacetic acid, then it would appear that both the cow and the sheep in late pregnancy converted the bulk of this factor into B-hydroxybutyric acid, by reduction, the remainder being decarboxylised to acetone. The cow at peak lactation, on the other hand, appeared to produce acetone and B-hydroxybutyric acid in about equal proportions from the acetoacetic acid, and so was able to form a higher percentage of iso-propanol.

On starving rats and a normal, but rather obese, human for ten days, Wicks et al (1940) noted a sudden rise in the blood ketones in the first 48 hours of starvation in the rat, and in the first 85 hours in the human, from a level of about 1 mg. acetone/100 ml. to 15 to 20 mg. acetone/100 ml. The latter level was maintained with minor fluctuations for the remaining days of the fast. They suggested that the rate of increase in the degree of ketosis at the onset of fasting was probably determined by the amount of stored carbohydrate and protein. The maintenance of ketosis at a more or less constant level may have been due to the availability for catabolism of a limited, but relatively constant, source/

source of carbohydrate from tissue protein and fat glycerol. Crandall (1940, 1941) starved both dogs and men for three days and found a state of ketosis was produced in both. The two species differed only in the rate of onset and intensity of ketonaemia. He found that the onset of ketosis in man was much more rapid, a ketonaemia being found 15 hours after the last meal. After 63 hours the average level of total ketones attained in the blood was 36.4 mg. B-hydroxybutyric acid/100 ml. (about 20 mg. acetone/ 100 ml.) as Wick et al noted. In the dog no ketonaemia appeared for 48 to 72 hours and then the increase in ketone body concentration was very slow.

It would appear from the literature that the dry non-pregnant cow, or one at low lactation, gives much the same response to starvation as the steer, viz., no appreciable rise in blood and urinary ketones. The cow at peak lactation or the cow or sheep in late pregnancy, however, responded to starvation in a manner entirely different from that found in the dry non-pregnant cow, steer, man, dor or rat, in that the level of total acetone bodies found in the blood was still rising after five days of fast. It was suggested by Wicks et al. (1940) that the stabilisation in the blood of the total ketone level attained after a few days in man and dog/

dog, etc., was due to the production of carbohydrate from tissue protein and fat. It would appear, therefore, that the fasting cow at peak lactation or cow or sheep in late pregnancy was unable to produce sufficient carbohydrate from these sources to maintain the blood sugar and keep down the acetone body levels, even with a drop in milk yield in the case of the lactating cow. This may be associated entirely with the greater demands for carbohydrate imposed by the foetus or by lactation even at the reduced level. Alternatively the mechanisms for bringing such compensatory changes into play may be less effective in the ruminant or the demands of the foetus or of a high milk yield may deplete the available reserves to such an extent as to hinder the attainment of equilibrium.

The chain of events suggested by the above observations is primarily the formation of aceto: acetate from the catabolism of fat and its rapid excretion or conversion into acetone and B-hydroxy: butyric acid. These various fractions gain the blood stream whence they pass into the milk and urine and also the rumen. In this organ there occurs the formation of iso-propanol, possibly from acetone, which then passes back into the blood stream and eventually reaches the milk and urine though at very low levels.

SUMMARY/

SUMMARY.

A detailed study of the fasting ketosis of dairy cows is described and the response obtained compared with that obtained in other species. All the acetone bodies studied, viz., acetone, aceto: acetic acid, B-hydroxybutyric acid and iso-propanol, showed a highly significant increase which was pre: dominantly linear in blood, milk and rumen contents. The first three appeared to originate in the tissues but the site of origin of the iso-propanol was most probably the rumen contents. The response of the blood sugar to fasting was found to be much the same as for other species while the ketonaemia appeared to be more marked.

The ketosis produced on complete starvation of a ewe in late pregnancy was found to be very similar to that produced in a cow in the same con: dition, and to differ from that produced in a cow at peak lactation in the proportions of the various acetone bodies present.

The significance of these findings is discussed.

IV. METABOLISM OF INDIVIDUAL KETONES.

INTRODUCTION.

Until very recently the work carried out involving administration of the various acetone bodies has been either to determine the part they played in diabetes and the diabetic coma in man and dog etc., or to elucidate the processes of fat metabolism in the animal body. In none of these investigations was the ruminant used as the exper: imental animal.

According to Frerichs (1883) Salomon fed acetone and acetoacetate to men and dogs and pro: duced no symptoms in his patients, but noted a slight acetonuria and a strong aromatic smell in the breath of human subjects after giving them 40 grms. of acetoacetate daily. Albertoni (1884) repeated these experiments with acetone and found 15 - 20 grms., given by mouth, caused no symptoms in men; 4 grms. per os produced a form of drunken: ness in dogs, and 8 grms. proved fatal, while 6 ml. injected into the jugular vein of a dog caused only temporary prostration. Sharz (1897) stated that normal dogs could metabolise acetoacetate completely, while depancreatised dogs excreted part in the form of acetone.

Geelmuyden (1900), Blum (1910) and Neubauer (1910) carried out experiments on the interrelation: ship of acetoacetate/

ship of acetoacetate and B-hydroxybutyric acid with regard to fat metabolism, by administering these substances subcutaneously, or per os, to men and dogs, and showed that acetoacetate was the primary product being readily reduced to B-hydroxybutyric acid by the body, and not vice-versa as was previously supposed.

Hurtley (1916) after reviewing all the previous work on the subject, and taking into account his own investigations, came to the conclusion that acetoacetic acid, being seven times stronger than B-hydroxybutyric acid as regards acidity, and a great deal more toxic, is the direct cause of diabetic coma, the conversion to B-hydroxybutyric acid being a protective mechanism.

Allen and Wishart (1923) repeated most of these experiments and expanded them. At intervals after the administration of various substances they carried out a Rothera test on the plasma and urine of the experimental rabbits and dogs they used. They obtained positive results in both immediately after an intravenous injection of acetone, and were surprised to obtain the same result twelve hours later. Immediately after the administration of acetoacetate to a rabbit by stomach tube, or intravenously, the plasma and urine both gave positive results very strongly/

strongly and very slightly respectively, while an intravenous injection to a dog only caused a slight acetonuria. Successive intravenous injections to a rabbit caused death; the animal died in a coma due to failure of the heart and respiration. B-hydroxy: butyric acid administration produced very little response when given intravenously to dogs, no acetone or acetoacetate were found in the plasma, or urine, though a rise in B-hydroxybutyric acid was noted in each. Urine giving a slightly positive Rothera reaction was excreted by a rabbit after being given B-hydroxybutyric acid by stomach tube.

Koffman (1937) and Holmes (1950) investigating bovine ketosis in dairy cattle gave cows acetone by mouth, and found that very large doses had a tem: porary toxic effect. The blood serum levels rose reaching a maximum value about an hour after administration. Holmes found that the acetone was rapidly excreted by the mammary gland, but could find no detectable amounts in the urine, his method of estimation being a modified form of Rotheras test.

As ruminant metabolism is often very different from that of single stomached animals, it was thought advisable to carry out experiments to investigate the chemical changes that occur, in the blood, rumen liquor, milk and urine, in both sheep and cows, when the ketone/

the ketone bodies, acetone, acetoacetic acid, B-hydroxybutyric acid and iso-propanol are administered, It was hoped that the results might throw some more light on the metabolism of these substances in the body.

METHOD.

Most of the administration experiments covered a period of three days; during that time the animals were given their normal feed, and an ample supply of water. The sheep were kept in metabolism cages, but the cows remained in their stalls. Twenty four hour samples of urine were taken whenever possible. During the summer months the cows were fed freshly cut grass.

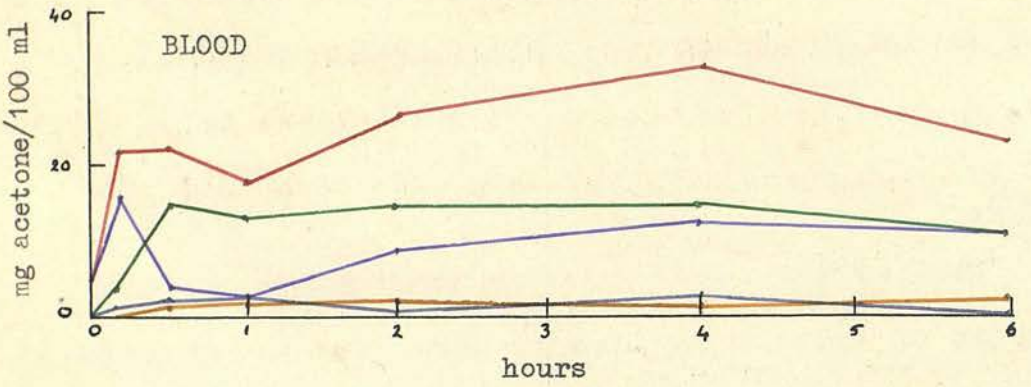
Access to the sheep's rumen was obtained by means of an ebonite rumen canula with a screw cap, or a self-sealing fistula. A long stomach tube was employed in the case of the cows, both for removing the rumen contents, and adding to them.

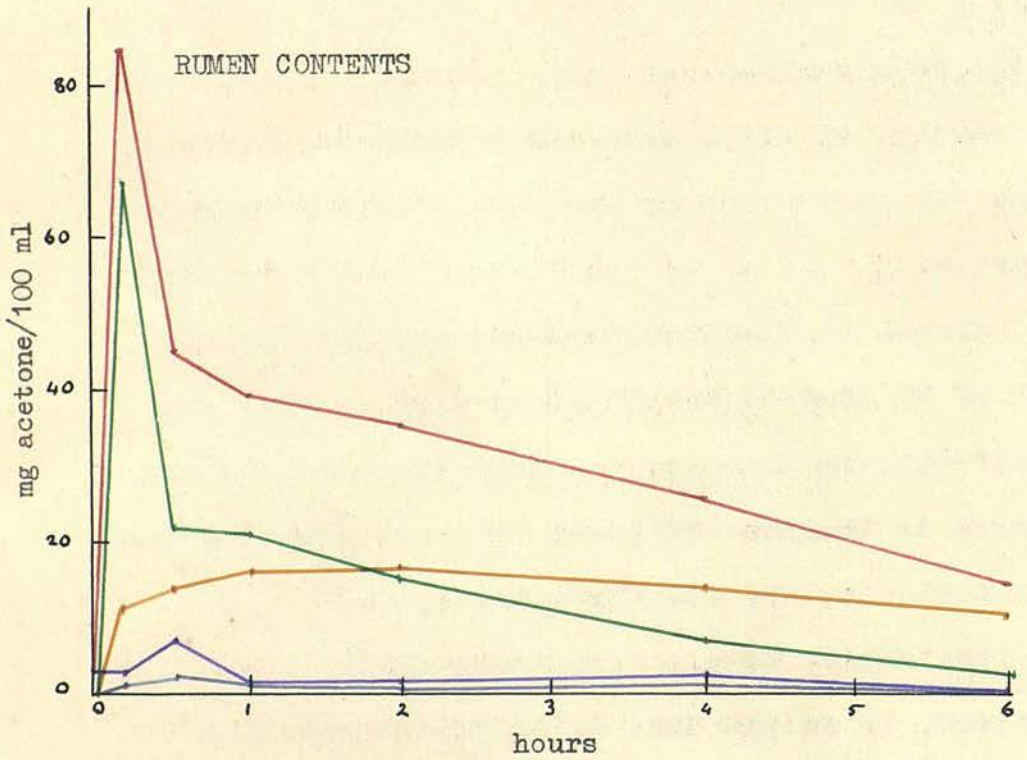
The ketone bodies were added to the rumen dissolved, or in the case of ethyl acetoacetate suspended, in a large volume of water, 1-3 litres for a cow and 100-200 ml. for the sheep. The ketone bodies in physiological saline, about 250-500 ml. for the cow, and 25-50 ml. for the sheep, were injected over a period of from 5 to 10 minutes into the jugular vein./

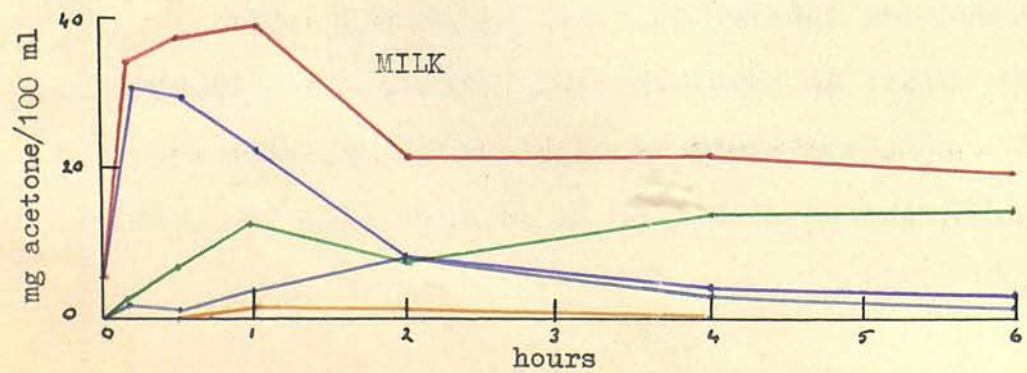
FIGURE 9.

The effect of injecting 100 ml. acetone into the rumen of a cow.

Total acetone bodies —, acetone —, acetoacetic acid —, β-hydroxy butyric acid —, iso-propanol —.







vein.

Before administration.and at certain periods after, viz., 10 mins., 40 mins., 1 hr., 3 hrs., 4 hrs., 6 hrs., and 24 hrs., blood, rumen contents and milk samples were taken. The blood was taken from the mammary vein in the cow, and the unused jugular vein in the sheep.

The ketone body estimations were carried out as previously described.

RESULTS.

I. Single Administration.

Normal animals were used in all these experiments, i.e., B-hydroxybutyric acid was the only acetone body present in the blood, rumen contents, milk and urine prior to administration.

A. The Administration of Acetone.

1. Into the rumen of a cow.

Ten minutes after the administration of 100 ml. acetone all the ketones, acetone, acetoacetic acid, B-hydroxybutyric acid and iso-propanol, were found in the rumen contents, and all but iso-propanol in the blood and milk, though an hour after administration iso-propanol was also found in both the latter. Within the first hour the levels of total acetone bodies and acetone/

TABLE 13.

Acetone Bodies after administration of 100 ml acetone into the rumen of a cow.

(As percentage of total)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
1. Blood.				
Acetone.....	0	19.08	75.72	57.21
Acetoacetic acid.....	0	4.71	7.07	1.65
B-hydroxybutyric acid.	100	76.21	8.52	32.53
Iso-propanol.....	0	0	8.69	8.61
2. Rumen Contents.				
Acetone.....	0	80.17	54.32	44.80
Acetoacetic acid.....	0	0.91	2.92	1.73
B-hydroxybutyric acid.	100	3.48	2.52	16.12
Iso-propanol.....	0	15.44	40.24	48.35
3. Milk.				
Acetone.....	0	3.34	32.82	29;78
Acetoacetic acid.....	0	5.76	10.39	31.99
B-hydroxybutyric acid.	100	90.90	56.39	34.29
Iso-propanol.....	0	0	0.40	3.94

and acetone in the rumen contents fell rapidly, the values found one hour after administration being less than half those found after ten minutes. Subsequently the total acetone bodies decreased much less rapidly approaching the normal value between twenty four and forty eight hours after administration. (Tables 13 and 31, and Fig. 9.). As the percentage content of acetone fell in the rumen contents, that of iso-propanol rose until it was the predominant acetone body present. The proportion of B-hydroxybutyric acid naturally fell with the appearance of the other acetone bodies, but gradually rose again attaining its original value of 100% about one to two days after administration. During the whole experiment only very small quantities of acetoacetic acid were found.

The concentrations of acetone and acetoacetic acid rose in the blood reaching maximum values about two hours after administration, while the content of B-hydroxybutyric acid fell during the first two or three hours, and then rose again until it was the only acetone body present. Iso-propanol was found in the blood an hour after administration and rose slightly in the next two hours, but at all times only a fraction/

TABLE 14.

Acetone Bodies after administration of 4 ml acetone to a sheep.

(As percentage of total)

	Time after administration			
	0	10 mins.	1 hr.	2 hrs.
A. Into the Rumen.				
1. Blood.				
Acetone.....	0	3.90	12.32	19.27
Acetoacetic acid.....	0	1.73	4.53	9.56
B-hydroxybutyric acid.	100	94.37	81.64	70.04
Iso-propanol.....	0	0	1.51	1.13
2. Rumen Contents.				
Acetone.....	0	92.25	43.23	48.34
Acetoacetic acid.....	0	0.45	15.86	17.16
B-hydroxybutyric acid.	100	4.28	26.38	20.74
Iso-propanol.....	0	3.02	14.53	13.76
B. Intravenously.				
1. Blood.				
Acetone.....	0	44.70	46.20	42.35
Acetoacetic acid.....	0	2.01	2.92	7.84
B-hydroxybutyric acid.	0	52.45	48.37	33.06
Iso-propanol.....	0	0.84	2.51	16.75
2. Rumen Contents.				
Acetone.....	0	0	27.46	4.10
Acetoacetic acid.....	0	0	10.07	7.84
B-hydroxybutyric acid.	0	80.10	38.82	23.86
Iso-propanol.....	0	19.90	23.65	64.20

fraction of that encountered in the rumen was found. In the milk the concentrations of acetone and acetoacetic acid reached maximum values three to four and two hours respectively, after administration. On the whole the percentage contents of acetoacetic acid and B-hydroxybutyric acid were higher than in the blood, while those of acetone and iso-propanol were lower.

All the acetone bodies were excreted in the urine within two hours of administration, acetone being the most prominent followed by B-hydroxybutyric acid.

2. Into the rumen of a sheep.

In this case 4 ml. acetone was injected into the rumen. Ten minutes after, as in the cow, all the acetone bodies were found in the rumen contents, and all but iso-propanol in the blood. The effects produced by the administration were in general the same as in the cow, but in both the blood and rumen contents the rise of acetoacetic acid was more marked, and that of iso-propanol proportionately less so. (Tables 14 and 31). B-hydroxybutyric acid was found to be the main excretory product in a twenty four hour sample of urine, with a small proportion of acetone and traces of acetoacetic acid and iso-propanol.

3. To a cow/

TABLE 15.

Acetone Bodies after administration of 75 ml acetone intravenously to a cow.

(As percentage of total).

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
1. Blood.				
Acetone.....	0	43.74	33.59	28.33
Acetoacetic acid.....	0	2.29	0.69	2.18
B-hydroxybutyric acid.	100	52.41	64.57	69.49
Iso-propanol.....	0	1.56	1.15	0
2. Rumen Contents.				
Acetone.....	0	9.92	16.30	18.02
Acetoacetic acid.....	0	0.71	4.77	1.70
B-hydroxybutyric acid.	100	80.71	44.61	25.23
Iso-propanol.....	0	8.66	34.32	55.05
3. Milk.				
Acetone.....	0	13.14	45.77	19.23
Acetoacetic acid.....	0	13.62	4.23	22.36
B-hydroxybutyric acid.	100	73.24	50.00	58.41
Iso-propanol.....	0	0	0	0

3. To a cow by intravenous injection.

Ten minutes after the injection of 75 ml. acetone all the acetone bodies, acetone, acetoacetic acid, B-hydroxybutyric acid and iso-propanol were found in the blood and rumen contents, and all but iso-propanol in the milk; this fraction was absent during the whole experiment. (Tables 15 and 32). The level of total acetone bodies in the blood did not decrease as rapidly as in the rumen in the previous experiment, (Fig. 10). The injected acetone disappeared more quickly, being replaced by B-hydroxybutyric acid, as the concentrations of acetoacetic acid and iso-propanol remained low, no traces of the latter being found two hours after injection.

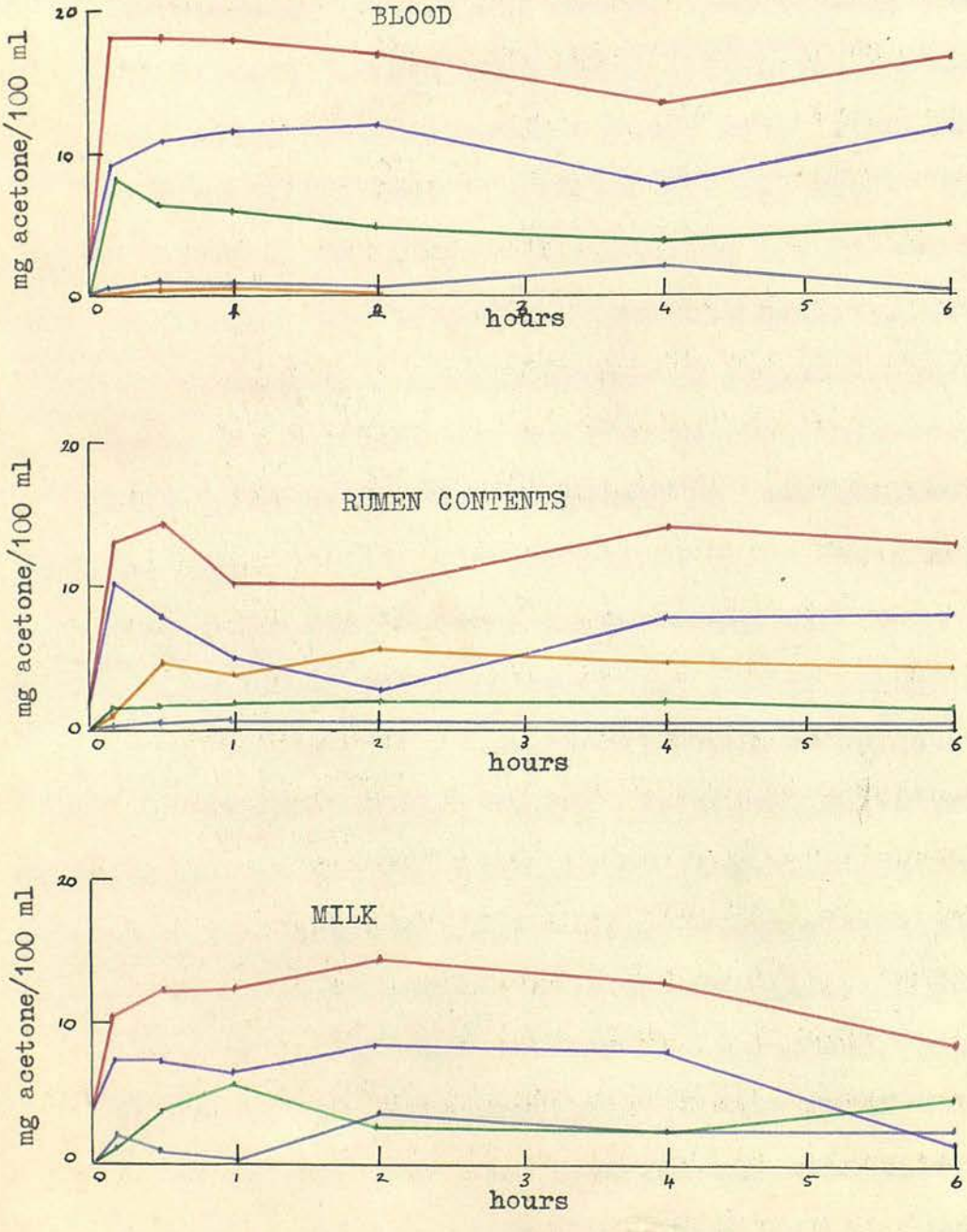
The concentrations of acetone and iso-propanol in the rumen contents rose steadily, reaching maximum values about two hours after injection, while the proportions of B-hydroxy: butyric acid fell during this period, but there: after rose again to the normal levels. During the whole experiment only traces of acetoacetic acid were found in the rumen contents.

As before, the proportions of the acetone bodies found in the milk were very similar to those in the blood, though the content of aceto: acetic acid was slightly higher. One hour after injection/

FIGURE IO.

The effect of administering 75 ml. acetone to a cow by intravenous injection.

Total acetone bodies —, acetone —, acetoacetic acid —, β-hydroxy butyric acid —, iso-propanol —.



injection the concentration of acetone had reached a maximum and B-hydroxybutyric acid a minimum.

While traces of both acetoacetic acid and iso-propanol were found in all the urine samples collected, the most prominent ketones were acetone and B-hydroxybutyric acid. These were excreted in about equal quantities one hour after injection, but thereafter increasingly less acetone and proportionately more B-hydroxybutyric acid was found.

4. To a sheep by intravenous injection.

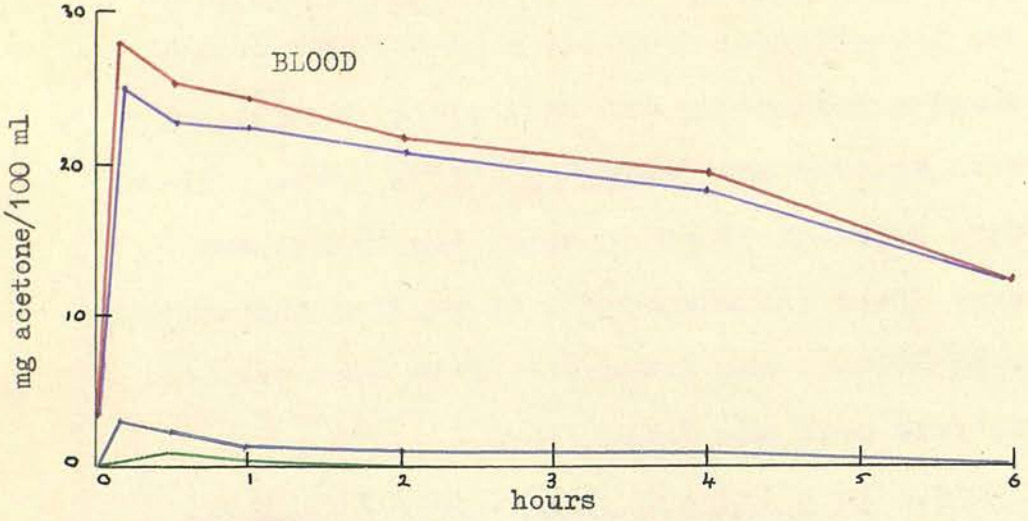
Ten minutes after the injection of 4 ml. acetone all the ketones were found in the blood, but only B-hydroxybutyric acid and iso-propanol in the rumen contents. (Table 14 and 32). An hour later acetone and acetoacetic acid were also found in the rumen. The concentration of iso-propanol rose in the blood and rumen contents reaching maximum values about two hours after injection, and then gradually fell again. The concentration of B-hydroxybutyric acid, on the other hand, fell during the first two hours and then rose. As before, the percentage of acetoacetic acid in both the blood and rumen contents was higher than in the cow.

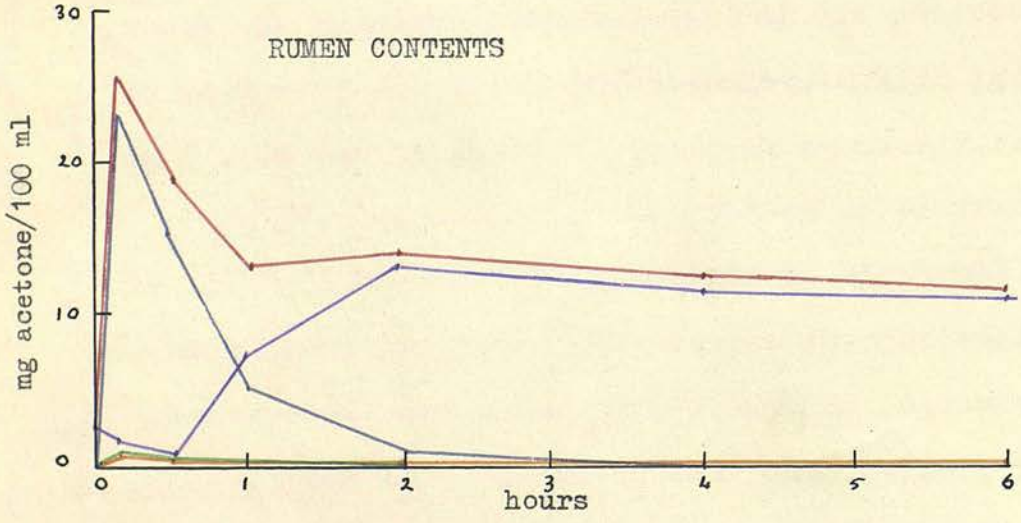
B. The Administration/

FIGURE II.

The effect of injecting 150 ml. ethyl acetoacetate into the rumen of a cow.

Total acetone bodies——, acetone——, acetoacetic acid——, β-hydroxy butyric acid——, iso-propanol——.





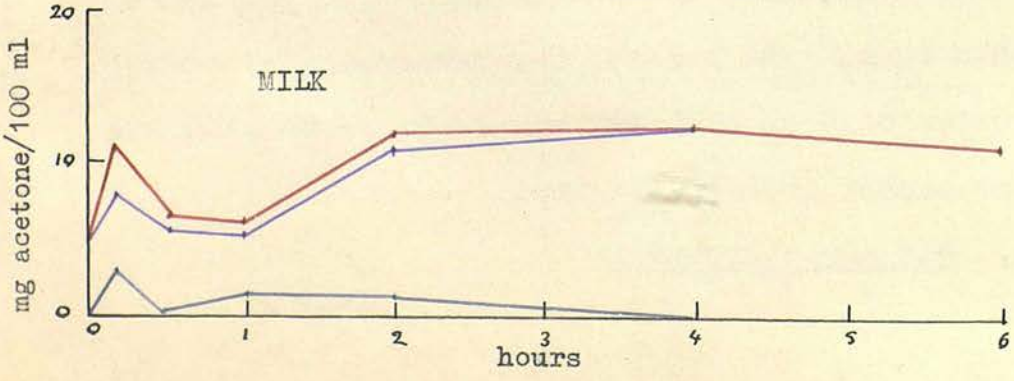


TABLE 16.

Acetone Bodies after administration of 150 ml ethyl acetoacetate into the rumen of a cow.

(As percentage of total).

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
1. Blood.				
Acetone	0	0	1.52	0.85
Acetoacetic acid	0	10.62	4.18	1.46
B-hydroxybutyric acid.	100	89.38	94.30	97.69
Iso-propanol	0	0	0	0
2. Rumen Contents.				
Acetone	0	2.15	2.69	0
Acetoacetic acid	0	90.99	38.77	1.79
B-hydroxybutyric acid.	100	4.87	54.05	96.06
Iso-propanol	0	1.99	4.49	2.15
3. Milk.				
Acetone	0	0	0	0
Acetoacetic acid	0	26.51	15.35	7.96
B-hydroxybutyric acid.	100	73.49	84.65	92.04
Iso-propanol	0	0	0	0

B. The Administration of Ethyl Acetoacetate.

1. Into the Rumen of a Cow.

The effect of injecting ethyl acetoacetate into the rumen was very much less marked than that produced when acetone was injected, either into the rumen or intravenously. (Fig. 11). Ten minutes after the administration of 150 ml. of ethyl acetoacetate all the acetone bodies were found in the rumen, but after two hours B-hydroxy: butyric acid and only a trace of acetoacetic acid and iso-propanol were present. (Tables 16 and 33). The fall in acetoacetic acid content was very rapid, and was accompanied by a less marked fall in acetone, which appeared immediately after the injection. Very small amounts of iso-propanol were found, the maximum concentration occurring an hour after administration. After a drop in concentration in the first ten minutes, there was a marked rise in B-hydroxybutyric acid which was the only ketone present six hours after injection.

Maximum concentrations of acetoacetic acid were found in the blood and milk ten minutes after administration, but in both cases the amounts present were small. Traces of acetone were found in the blood/

TABLE 17.

Acetone Bodies after administration of 4 ml ethyl acetoacetate into the rumen of a sheep.

(As percentage of total)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
1. Blood.				
Acetone.....	0	0	10.30	0
Acetoacetic acid.....	0	0	27.20	0
B-hydroxybutyric acid.	0	100	62.50	100
Iso-propanol.....	0	0	0	0
2. Rumen Contents.				
Acetone.....	0	13.37	5.57	0
Acetoacetic acid.....	0	86.18	85.66	0
B-hydroxybutyric acid.	0	0	1.75	83.77
Iso-propanol.....	0	0.45	7.02	16.23

in the blood after an hour, but not in the milk, and throughout the whole experiment no iso-propanol was found in either.

Two hours after administration all the acetone bodies were excreted in the urine, acetoacetic acid and B-hydroxybutyric acid being the most prominent and present in about equal amounts. Subsequent samples contained higher proportions of B-hydroxybutyric acid and only traces of acetoacetic acid. Twenty four hours after administration the excretion was normal, only B-hydroxybutyric acid being present. During the first six hours following administration acetoacetic acid was excreted in the animal's breath.

2. Into the rumen of a sheep.

The effects of injecting 4 ml. ethyl acetoacetate into a sheep's rumen were very similar to those in the cow, (Tables 17 and 33), though the fall in acetoacetic acid was more marked, none being found in either the rumen contents or the blood two hours after administration. No aceto: acetic acid was found in the urine, B-hydroxy: butyric acid only being excreted in a twenty four hour sample.

3. To a cow/

3. To a cow by intravenous injection.

30 ml. ethyl acetoacetate injected intravenously into the cow had very little effect on the animal, though the breath smelt strongly of acetoacetic acid. Ten minutes and one hour after injection traces of acetone were found in the blood, and after the former time interval the equivalent of 4.84 mg. acetone/100 ml. of acetoacetic acid, along with B-hydroxybutyric acid in normal amounts. Both acetone and acetoacetic acid were found in the milk during this period, but only acetoacetic acid in the rumen contents.

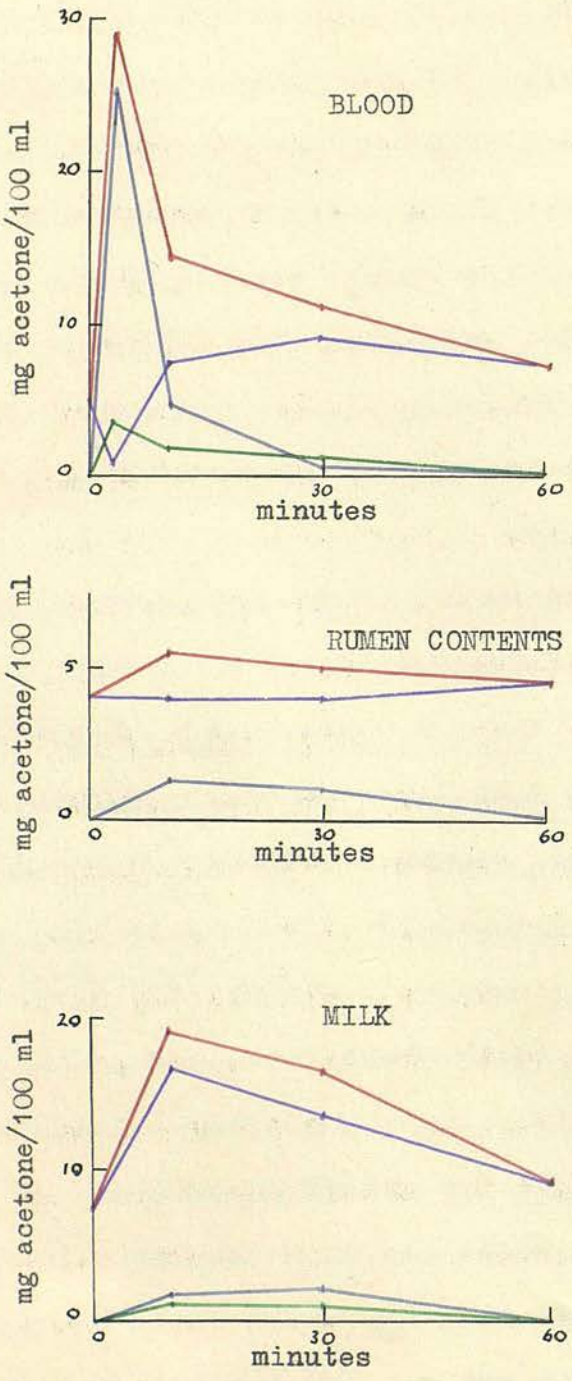
75 to 80 ml. ethyl acetoacetate when injected intravenously acted as a temporary anaesthetic, the cow remaining unconscious for two to three minutes. On regaining consciousness breathing became difficult and the animal coughed a great deal. The smell of acetoacetic acid was very marked. One animal, which was particularly badly affected, developed pneumonia a few days after the experiment. Ten minutes after injection the amount of acetoacetic acid found in the blood and milk was about the same as above. It was impossible to take a rumen sample at this time as the animal's breathing

was so/

FIGURE I2.

The effect of administering 80 ml. ethyl acetoacetate to a cow by intravenous injection.

Total acetone bodies——, acetone——, acetoacetic acid——, β-hydroxy butyric acid——, iso-propanol——.



was so disturbed, but one hour after the injection traces of acetoacetic acid were found in the rumen contents. In one cow a blood sample was taken about three minutes after the injection (Fig. 12), just as the animal regained consciousness, 3.15 mg. acetone/100 ml, the equivalent of 25.14 mg. acetone/100 ml. of acetoacetic acid and 0.62 mg. acetone/100 ml. of B-hydroxybutyric acid were found to be present. The cow micturated naturally ten to fifteen minutes after the injection and the urine contained 3.66 mg. acetone/100 ml., the equivalent of 30.17 mg. acetone/100 ml. of acetoacetic acid and 32.59 mg. acetone/100 ml. of B-hydroxybutyric acid.

4. To a sheep by intravenous injection.

4 or 5 ml. of ethyl acetoacetate injected intravenously into the sheep had, like 30 ml. to a cow, very little effect on the animal. The rise in total acetone bodies in the blood was negligible, and the maximum amount of aceto: acetic acid found in the blood was 1 mg. acetone/ 100 ml. ten minutes after injection. No acetone or iso-propanol were found, nor was there any change in the rumen contents. There was, however, a strong smell of acetoacetic acid in the animal's/

the animal's breath.

When 10 ml. acetoacetate were injected the effects were clinically more marked, the animal collapsed for a few seconds, and for some time after the breathing was laboured. As before the breath smelt strongly of aceto: acetic acid. Chemically the position was much the same, but ten minutes after injection the equivalent of 2 mg. acetone/100 ml. of aceto: acetic acid were found in the blood.

C. The administration of sodium-B-hydroxybutyrate.

1. Into the rumen of a cow.

100 grm. of sodium B-hydroxybutyrate were injected into a cow's rumen with negligible results. The concentration of B-hydroxybutyric acid in the rumen rose immediately and then gradually decreased, regaining normal values about twenty four hours later. No other acetone bodies were found throughout the whole experiment in either the rumen contents, blood, milk or urine. There was a slight rise in the B-hydroxy: butyric acid content of the blood and milk in the first hour, but the urinary excretion was not noticeably changed.

2. Into the rumen of a sheep.

The results obtained after injecting 10 grm. sodium/

FIGURE 13.

The effect of injecting 250 ml. iso-propanol into the rumen of a cow.

Total acetone bodies——, acetone——, acetoacetic acid——, β-hydroxy butyric acid——, iso-propanol——.

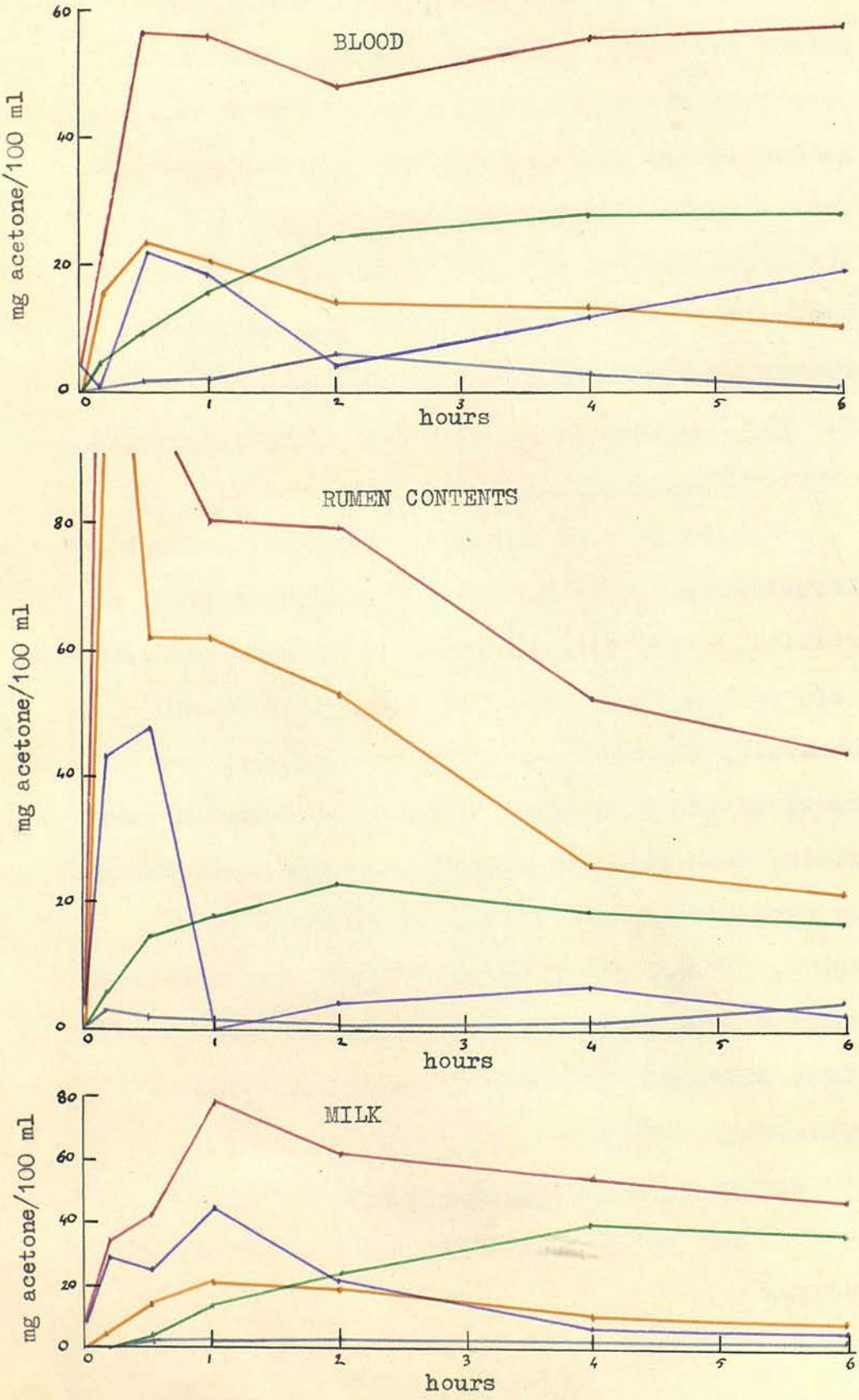


TABLE 18.

Acetone Bodies after administration of 250 ml iso-propanol into the rumen of a cow.

(as percentage of total)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
1. Blood.				
Acetone.....	0	21.84	27.55	42.94
Acetoacetic acid.....	0	1.83	2.36	10.21
B-hydroxybutyric acid.	100	3.53	34.08	22.01
Iso-propanol.....	0	72.80	36.01	24.84
2. Rumen Contents.				
Acetone.....	0	1.80	20.97	28.52
Acetoacetic acid.....	0	1.20	0.72	1.10
B-hydroxybutyric acid.	100	14.37	0.25	4.48
Iso-propanol.....	0	82.63	78.06	65.90
3. Milk.				
Acetone.....	0	0	15.89	33.52
Acetoacetic acid.....	0	0	1.01	4.16
B-hydroxybutyric acid.	100	86.31	56.94	32.53
Iso-propanol.....	0	13.69	26.16	29.79

sodium B-hydroxybutyrate into the rumen of a sheep, were similar to those in the cow.

3. To a sheep by intravenous injection.

As after administration into the rumen, only B-hydroxybutyric acid was found in the blood, rumen contents, and urine subsequent to the intravenous injection of 4 grm. sodium B-hydroxybutyrate. Even ten minutes after the injection no appreciable rise in the B-hydroxybutyric acid content of the blood was noted, and there was none in the rumen contents or urine.

D. The administration of iso-propanol.

1. Into the rumen of a cow.

All four acetone bodies were found in the blood and rumen contents ten minutes after the administration of 250 ml. iso-propanol into the rumen, (Fig. 13). After the same time interval only B-hydroxybutyric acid and iso-propanol were found in the milk, but all four ketones were present one hour after administration (Tables 18 and 34). The percentage of iso-propanol in the rumen fell steadily, though not rapidly, and had all disappeared between forty eight and seventy two hours after the administration. There was very little change in the percentage content of acetoacetic acid throughout the experiment/

TABLE 19.

Acetone Bodies after administration of iso-propanol to a sheep.

(as percentage of total)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
A. 15 ml. into the Rumen.				
1. Blood.				
Acetone.....	0	15.05	37.07	37.07
Acetoacetic acid.....	0	11.31	7.59	6.40
B-hydroxybutyric acid.	100	26.53	41.63	52.02
Iso-propanol.....	0	47.11	13.71	4.51
2. Rumen Contents.				
Acetone.....	0	1.22	4.40	10.37
Acetoacetic acid.....	0	0.45	1.01	2.01
B-hydroxybutyric acid.	100	0.28	11.59	17.81
Iso-propanol.....	0	98.05	83.00	69.81
B. 4 ml. Intravenously.				
1. Blood.				
Acetone.....	0	0	55.08	64.82
Acetoacetic acid.....	0	0	4.07	11.58
B-hydroxybutyric acid.	100	33.34	7.55	1.74
Iso-propanol.....	0	66.66	33.30	21.86
2. Rumen Contents.				
Acetone.....	0	0	9.19	35.21
Acetoacetic acid.....	0	0	15.85	3.25
B-hydroxybutyric acid.	100	66.22	0	5.26
Iso-propanol.....	0	33.78	74.90	56.28

experiment, while that of B-hydroxybutyric acid fell in the first hour and then rose again. As the concentration of iso-propanol fell that of acetone rose, reaching a maximum value about three hours after administration, and then decreasing again till it disappeared about three days later. Ten minutes after administration appreciable quantities of iso-propanol and acetone were found in the blood. The concentration of the latter increased rapidly in the first six hours and then gradually fell again, while maximum concentrations of the former were noted one hour after administration. The concentration of B-hydroxybutyric acid rose in the first two hours and then gradually decreased.

Iso-propanol was rapidly excreted in the milk and urine, though in both B-hydroxybutyric acid was present in the highest concentrations. After the first hour relatively large quantities of acetone were found in the milk, and the amount excreted in the urine exceeded the iso-propanol. Only traces of acetoacetic acid were found in both.

2. Into the rumen of assheep.

As before the response of the sheep was very similar to that of the cow. (Tables 19 and 34). In this case 15 ml. were injected into the rumen/

TABLE 20.

Acetone Bodies after administration of 125 ml. iso-propanol intravenously to a cow.

(As percentage of total)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
1. Blood...				
Acetone.....	0	3.86	43.51	57.64
Acetoacetic acid.....	0	4.31	7.20	1.23
B-hydroxybutyric acid.	100	46.14	3.47	23.40
Iso-propanol.....	0	45.69	45.82	17.73
2. Rumen Contents.				
Acetone.....	0	8.76	16.80	22.76
Acetoacetic acid.....	0	6.76	4.33	5.54
B-hydroxybutyric acid.	100	7.20	0.62	14.51
Iso-propanol.....	0	77.28	78.25	57.19
3. Milk.				
Acetone.....	0	33.80	39.83	40.30
Acetoacetic acid.....	0	6.20	9.30	10.42
B-hydroxybutyric acid.	100	4.00	5.80	18.65
Iso-propanol.....	0	56.00	45.07	30.63

rumen. On the average the percentage content of acetoacetic acid in the blood was higher, and that of iso-propanol lower, than in the cow, and the fall in percentage content of iso-propanol and rise in acetone in the rumen was not so rapid.

3. To a cow by intravenous injection.

Ten minutes after the injection of 125 ml. iso-propanol all the acetone bodies were found in the blood, rumen contents and milk. (Tables 20 and 35). The steady decrease of iso-propanol in the blood was accompanied, during the first two hours, by a rise in acetone content. Very little acetoacetic acid was found. After one hour steadily increasing percentages of B-hydroxy: butyric acid were noted. The percentage of iso-propanol found in the rumen, even ten minutes after injection, was higher than that in the blood, and fell less rapidly. In the blood the decrease in concentration was accompanied by a rise in acetone. The percentage content of acetoacetic acid remained fairly steady, while that of B-hydroxybutyric acid rose after the first hour.

The percentage of iso-propanol excreted in the milk was greater than in the previous experiment/

experiment, and was about equalled by acetone. The percentage of acetoacetic acid was slightly higher, and B-hydroxybutyric acid slightly lower than in the blood. In the first six hours after injection haemoglobin was found in the urine. The first sample, taken about one hour after administration, contained the equivalent of 21.53 mg. acetone/100 ml. of B-hydroxybutyric acid and 15 mg. acetone/100 ml. of iso-propanol with 7.65 mg. acetone/100 ml. and traces of acetoacetic acid. Later samples contained decreasing amounts of iso-propanol and increasing proportions of acetone. In all the samples B-hydroxybutyric acid was the main excretory product.

4. To a sheep by intravenous injection.

Ten minutes after the injection of 4 ml. iso-propanol only that substance and B-hydroxybutyric acid were found in the blood and rumen contents, though one hour later all four ketones were observed in both. (Tables 19 and 35). After the first hour the chain of events was very similar to that in the cow, though a twenty four hour sample of urine contained mainly B-hydroxybutyric acid, followed by iso-propanol, acetoacetic acid and acetone, in that order.

II. Continuous/

II. Continuous Administration.

Acetone and iso-propanol, when administered to the ruminant, produced the greatest response. As iso-propanol is formed in the rumen, and acetone could conceivably be formed in the body from aceto: acetic acid and fat metabolism, it was decided to administer iso-propanol, (into the rumen), and acetone and acetoacetate (intravenously), more or less continuously for several days to try and produce an experimental ketosis.

Owing to the mechanical difficulties involved when large experimental animals are used, the con: stant drip method of continuous administration was not utilised. Instead the animals were given two or four injections a day, the number depending on the tractability of the animal used. Samples were taken daily just before the first injection of that day.

Repeated injections of acetone intravenously.

1. To a cow.

As would be expected from the results of the previous experiments, there was a steady daily rise in the concentrations of acetone and acetoacetic acid in the blood, though the value of the total acetone bodies remained much the same over the administration period. (Table 21).

The percentage/

TABLE 21.

Acetone Bodies after administration of acetone intravenously twice a day to a cow.

(As percentage of total)

	Days of Experiment.					
	0	1	2	3	4	5
Acetone injected.	2 x 50 ml.	2 x 75 ml.	2 x 75 ml.			
1. Blood.						
Acetone.....	0	25.40	44.79	72.71		33.68
Acetoacetic acid.....	0	2.73	5.19	7.36		32.63
B-hydroxybutyric acid	100	70.41	45.95	17.22		33.69
Iso-propanol.....	0	1.46	4.07	2.71		0
Total Acetone Bodies (mg. acetone/100 ml.)....	2.71	20.55	24.11	22.28		6.68
2. Rumen Contents.						
Acetone.....	0	21.59	35.74	21.66		15.74
Acetoacetic acid.....	0	1.64	13.07	11.33		38.19
B-hydroxybutyric acid.	100	24.99	20.40	53.49		8.17
Iso-propanol.....	0	51.78	30.79	13.54		37.90
Total Acetone Bodies (mg. acetone/100 ml.)....	1.72	7.92	21.43	39.89		3.43
3. Milk.						
Acetone.....	0	16.07	48.88	63.29		56.84
Acetoacetic acid.....	0	7.84	21.64	29.35		22.81
B-hydroxybutyric acid	100	76.09	24.25	0.05		20.35
Iso-propanol.....	0	0	5.23	7.31		0
Total Acetone Bodies (mg. acetone/100 ml.)....	12.30	15.44	22.86	24.63		8.11

The percentage content of B-hydroxybutyric acid fell and that of iso-propanol remained low over the same period. Though the total acetone bodies rose steadily in the rumen over the administration period, the percentage contents of the constituent fractions varied from day to day. In general the content of acetone and acetoacetic acid rose while that of iso-propanol fell steadily. As in the blood there was a steady increase in the acetone and acetoacetic acid contents in the milk; in addition the total acetone bodies and iso-propanol content rose while B-hydroxybutyric acid fell.

Though all the acetone bodies were found in the urine after the first day of administration, B-hydroxybutyric acid was present in much the greatest proportions. On the third day, however, acetone and B-hydroxybutyric acid were found in about equal amounts.

2. To a sheep.

In general the response of the sheep was very similar to that of the cow, (Table 36). Slightly higher concentrations of acetoacetic acid were found in the blood, and over the period of administration this factor did not vary much. The proportions of iso-propanol in the rumen were lower/

TABLE 22.

Acetone Bodies after administration of iso-propanol into the rumen of a cow four times daily.

(As percentage of total)

	Days of Experiment.									
	0	1	2	3	4	5	6	7	8	9
Iso-propanol.	4 x 25 ml.	4 x 30 ml	4 x 35 ml.	3 x 50 ml	4 x 75 ml					
1. Blood.										
Acetone.....	34.79	55.11	58.75	55.95	53.68	73.06		50.32	11.34	9.69
Acetoacetic acid.....	2.93	15.50	10.31	10.50	9.57	4.05		11.10	5.98	3.59
B-hydroxybutyric acid....	61.76	26.52	23.86	24.38	28.32	0.39		35.06	82.68	86.72
Iso-propanol.....	0.52	2.87	7.08	2.17	8.43	22.50		3.52	0	0
Total Acetone Bodies (mg. acetone/100 ml.)....	22.76	26.13	38.30	49.07	48.62	62.22		25.04	15.87	13.93

TABLE 22 (Contd).

Acetone Bodies after administration of iso-propanol into the rumen of a cow four times daily.

(As percentage of total)

	Days of Experiment.									
	0	1	2	3	4	5	6	7	8	9
Iso-propanol injected.	4 x 25 ml.	4 x 30 ml.	4 x 35 ml.	3 x 50 ml.	4 x 75 ml.					
2. Rumen Contents.										
Acetone.....	28.72	38.21	43.88	23.29	14.47	35.33		24.24	31.81	20.93
Acetoacetic acid.....	2.02	3.06	1.95	1.35	0.91	4.48		17.47	3.64	7.75
B-hydroxybutyric acid....	40.54	15.00	3.67	3.56	6.00	4.49		7.18	13.45	49.62
Iso-propanol.....	28.72	43.73	50.50	71.80	78.62	55.70		51.10	51.10	21.70
Total Acetone Bodies (mg. acetone/100 ml.)....	18.80	23.55	28.71	59.89	139.87	94.24		21.53	18.39	6.45

TABLE 22 (Contd).

Acetone Bodies after administration of iso-propanol into the rumen of a cow four times daily.

(As percentage of total).

	Days of Experiment.									
	0	1	2	3	4	5	6	7	8	9
Iso-propanol injected.	4 x 25 ml.	4 x 30 ml.	4 x 35 ml.	3 x 50 ml.	4 x 75 ml.					
3. Milk.										
Acetone.....	51.80	46.92	54.34	45.49	29.88	53.49		35.23	15.23	0
Acetoacetic acid.....	1.30	7.91	0.32	2.33	10.95	13.79		1.49	19.35	53.98
B-hydroxybutyric acid....	46.90	43.15	41.52	46.74	50.09	23.12		60.36	55.42	46.02
Iso-propanol.....	0	2.02	3.82	5.44	9.08	9.59		2.92	0	0
Total Acetone Bodies (mg. acetone/100 ml.)....	20.80	29.73	49.78	64.30	90.37	88.68		37.71	13.33	8.54

lower than in the cow, while those of acetone were higher.

Repeated injections of iso-propanol into the rumen.

1. Of a cow.

Four days before the experiment on the cow, the results of which are shown in Table 22, the animal was subjected to a very heavy dosage of iso-propanol, given into the rumen, 400 ml. one day and 250 ml. the next. The value for total acetone bodies was too high to estimate with any degree of accuracy, so the cow was left for four days before repeating the experiment using smaller doses of iso-propanol. Even at the end of the four days appreciable quantities of the acetone bodies were still found in the blood, rumen contents and milk.

The administration of iso-propanol brought about a steady rise in the concentrations of acetone and total acetone bodies in the blood, rumen contents and milk, which gradually fell again when the injections ceased. As before the percentage content of acetoacetic acid in all three remained low during the whole experiment. The concentrations of iso-propanol found in the blood and milk were very much lower than in the rumen/

rumen contents, while those of acetone were much higher. The percentage contents of all the factors were very similar in the blood and milk.

2. Of a sheep.

The results given in Table 37 for the sheep show a greatly decreased response to the injections compared to the cow, while those in Table 37a are comparable. The experiment quoted in the latter table was carried out when the experimental sheep were relatively fresh, while the sheep in the experiment shown in Table 37 had been used for administration experiments for two years, and had possibly become accustomed to acetone bodies, so were able to metabolise them more readily.

Repeated injections of ethyl acetoacetate intravenously to a cow.

Owing to the toxicity of ethyl acetoacetate it was not thought advisable to inject more than 30 ml. at a time, so this dose was given four times daily. When morning samples were taken, just before the first injection of the day, no change from the normal was noted. On the third day samples were taken just before the third and fourth injections of the day, A, and B. respect: ively in Table 38. On both occasions traces of acetone/

acetone bodies were found in the blood, rumen contents, milk and urine. The percentage content of acetoacetate in both the blood and rumen was low, though the level was slightly higher in the latter. In all four body fluids, viz., blood, rumen contents, milk and urine, B-hydroxybutyric acid was the most prominent acetone body present. In sample A. iso-propanol was only found in the rumen contents, but in B. all the fluids contained traces of this fraction. Though the percentage of acetone in the blood and rumen contents exceeded that of acetoacetic acid, the reverse held true in the urine.

DISCUSSION.

The effects of administering the various acetone bodies to sheep and cows were the same qualitatively though not quantitatively. It is only natural that the exact amounts of a specific acetone body should vary between species as between individuals, though the overall picture may remain the same. In all cases the fall in concentration of the administered substance was very rapid, normal values being regained within about two days.

The cows used were not milked out at each sampling so the results given for the milk are cumulative/

cumulative over twelve hours. As in all cases the total, and individual, acetone bodies fell within about six hours of administration, either the acetone bodies were utilised by the mammary gland for energy purposes, or a back diffusion into the blood stream took place. Shaw (1942) has shown that the mammary gland can utilise B-hydroxybutyric acid, but not acetoacetic acid, for energy purposes. As acetoacetic acid was found in higher concentrations in the milk than in the blood, this would seem to indicate non-utilisation of this substance by the gland, accompanied possibly by inability to diffuse back into the blood.

From the figures given it is obvious that the total amount of acetone bodies excreted in the milk and urine in no way accounted for all the specific acetone body administered. The remainder of the acetone bodies, in one form or another, are probably completely metabolised in the body to carbon dioxide and water.

Prior to 1949 the general opinion appears to have been that acetone was inert in the body. Scharz (1897) stated that dogs could not oxidise acetone in the body, and Dye and McCandless in 1948 suggested that acetone was formed in the ruminant tissues, and body fluids, from the other acetone bodies, viz., B-hydroxybutyric/

B-hydroxybutyric acid and acetoacetic acid, and being relatively inert was ultimately excreted per se. The above experiments have shown that this is not true; acetone is by no means inert in the ruminant body, but is rapidly metabolised with the formation of all the other acetone bodies, viz., acetoacetic acid, B-hydroxybutyric acid and isopropanol. Plaut and Lardy (1950) carried out experiments with labelled acetone which showed that this substance could be incorporated into aceto: acetic acid by rat liver slices. As the rise in concentration of these three acetone bodies does not fully compensate for the fall in acetone concentration, it would appear that acetone is also metabolised in a slightly different manner without the production of the other acetone bodies. Barnes and Gurin (1948) stated that acetate and acetoacetate can condense with oxaloacetate to form citric acid, and so be utilised via the tricarboxylic acid cycle. Price and Rittenberg (1948) administered acetone with labelled carbon in a methyl group, and found that labelled carbon dioxide was excreted in the animal's breath, and labelled acetyl compounds in the urine; they concluded that the rat could actively metabolise acetone. Sakami (1950) after administering radio: active acetone to rats suggested, as a pathway of acetone utilisation, cleavage of the carbon chain into acetate/

acetate and formate, or substances derived from these compounds. Whether these results can be applied to the ruminant or not remains to be seen, but probably very similar actions take place in the ruminant tissues.

It is surprising to find that Holmes (1950) noted no acetonuria after feeding cows large quantities of acetone. In the above experiments, where much smaller amounts of acetone were administered, sufficient was found in the urine to give an intense colouration in a Rothera test. Within an hour of administration a high percentage of acetone was excreted, suggesting that this substance has a low renal-threshold value.

Plaut and Lardy, (1950) noted a rise in the B-hydroxybutyric acid and acetoacetic acid content of various tissues and in the urine, when acetone was metabolised in the rat. We noted a similar rise in the ruminant, and it would appear that the power of the tissues to metabolise acetone bodies is the same in the ruminant as in other animals.

It appears that the ruminant can metabolise acetone in the rumen, as well as in the blood and tissues with the formation of the other acetone bodies. It is interesting to note that iso-propanol is found in highest/

in highest concentrations in the rumen, and that in general this substance is found in the rumen before it is found in the blood,but never found in the blood before appearing in the rumen. This indicates that the site of formation of iso-propanol is in the rumen, and is probably due to bacterial fermentation. As iso-propanol appeared in the highest concentrations after the administration of acetone, it is reasonable to suppose that acetone can be converted directly into iso-propanol in the rumen, by reduction.

When iso-propanol was administered, either into the rumen or intravenously, large quantities of acetone were formed indicating that the above reaction, in the rumen, was reversible, and that iso-propanol could be oxidised to acetone in the blood stream and tissues.

A large proportion of the acetoacetate injected into the rumen formed B-hydroxybutyric acid indicating that this reduction was more easily accomplished than decarboxylation to acetone, which also took place. In general the response of the ruminant to acetoacetate administration per os was similar to that observed by Frerichs etc. in man and the dog - i.e., no symptoms but a strong smell of acetoacetic acid in the breath.

When/

When injected intravenously acetoacetic acid formed both acetone and B-hydroxybutyric acid, though in very small amounts. An enzyme has been demonstrated in Clostridium acetobutylicum which catalyses the decarboxylation of acetoacetic acid to acetone, (Johnson et al 1933), and Grégoire (1933) and Rossi (1938) claim to have found a similar catalyst in dog blood. These catalysts may well occur in the rumen and blood of the ruminant.

Acetoacetic acid disappeared from the blood remarkably quickly, possibly explaining why Allen and Wishart obtained no reaction with a Rothera test. The main methods of excretion were the quickest, viz., through the lungs and urine. Large doses of acetoacetic acid caused congestion of the lungs, with possible injury to the lung tissue. From the results it appeared that, as has been suggested, acetoacetic acid was very toxic to the ruminant, and in concentrations above about 25 mg. acetone/100 ml., in the cow, can produce a coma. This lends support to Hurtley's suggestion that a specific poisoning by acetoacetic acid causes the coma often observed in diabetes. The listlessness noted in ruminants suffering from bovine ketosis and pregnancy toxaemia, might be explicable on the same basis. Hurtley also/

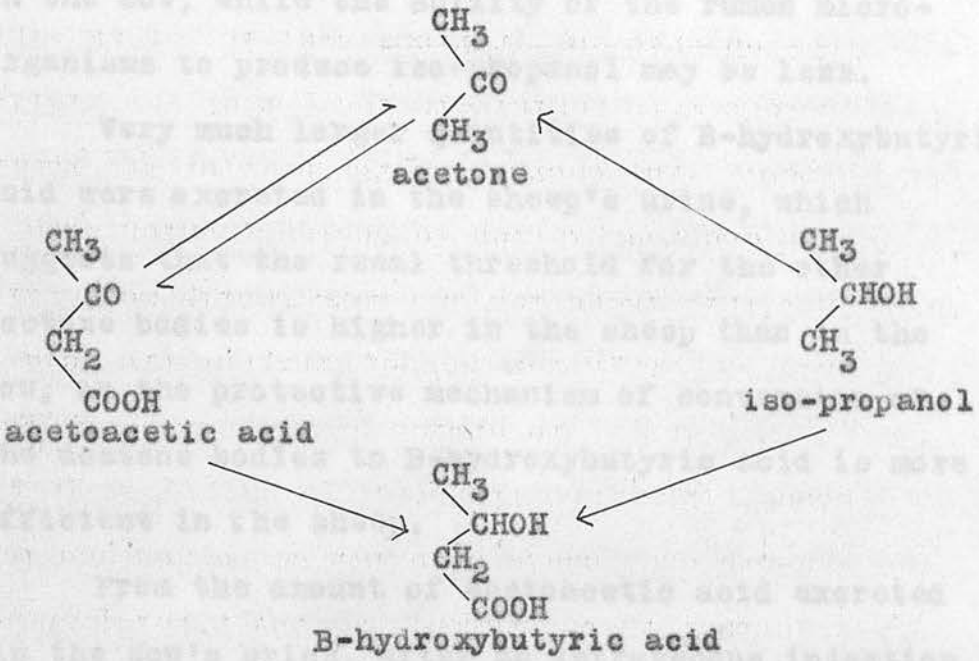
also suggested that the reduction of acetoacetate to B-hydroxybutyric acid was a protective mechanism delaying the coma. In all the previous experiments B-hydroxybutyric acid was formed from the other acetone bodies, and was the only acetone body present before, and after, the experimental period. This, taken with the fact that administration of B-hydroxybutyrate, either into the rumen or intravenously, produced none of the other acetone bodies, indicates that B-hydroxybutyric acid is the end point in acetone body conversion. As this substance is present in the normal animal it is obviously harmless, and so conversion to B-hydroxybutyric acid could be taken as a protective mechanism.

These experiments would also suggest that if in the ruminant, as Geelmuyder etc., found in man and dog, acetoacetic acid is formed in fat metabolism, it is the primary product being readily reduced to B-hydroxybutyric acid.

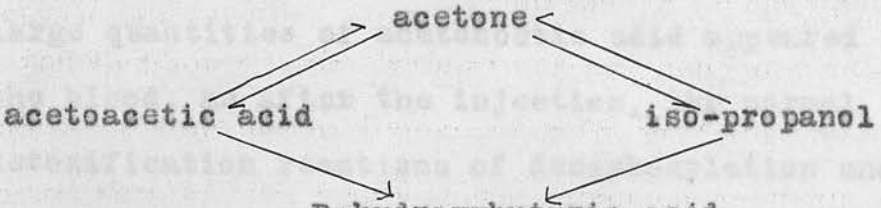
On the basis of the above results we would tentatively suggest the following as a possible cycle of events in the inter-acetone body conversions.

In the blood and tissues/

In the blood and tissues:-



In the rumen:-



There appears to be a slight difference in the response of the two ruminant species to acetone body administration. In nearly all cases higher concentrations of acetoacetic acid, and lower concentrations of iso-propanol were found in the sheep. The tolerance to acetoacetic acid in the sheep may be greater than in the cow/

in the cow, while the ability of the rumen micro-organisms to produce iso-propanol may be less.

Very much larger quantities of B-hydroxybutyric acid were excreted in the sheep's urine, which suggests that the renal threshold for the other acetone bodies is higher in the sheep than in the cow, or the protective mechanism of conversion of the acetone bodies to B-hydroxybutyric acid is more efficient in the sheep.

From the amount of acetoacetic acid excreted in the cow's urine, after an intravenous injection of acetoacetate, it appears that the renal threshold for this substance in the cow is very low. This would be an additional protective measure; if large quantities of acetoacetic acid appeared in the blood, as after the injection, the normal detoxification reactions of decarboxylation and reduction, to acetone and B-hydroxybutyric acid respectively, would not be able to act with sufficient speed to prevent the toxic action, without the excretion of a large proportion of the acetoacetic acid through the urine taking place at the same time.

In two cases of repeated administration, acetone intravenously and iso-propanol into the rumen, a form of ketosis was produced, though the relative proportions/

proportions of the acetone bodies varied somewhat. The proportion of acetone found in the blood and rumen contents in both experiments was about the same, while that of acetoacetic acid remained low during the experimental period. The main difference lay in the proportion of iso-propanol which was naturally higher in both the blood and rumen contents in the experiment in which it was administered. The proportion of iso-propanol found in the sheep's rumen was lower than in the cow's, while that of acetone was higher.

Repeated injections of acetoacetate produced a very transient ketosis after each injection. The body mechanisms were able to detoxify, and completely eliminate, 30 ml. doses of acetoacetate within about three hours, and only a very slight accumulative effect was noted in the percentage contents of the various factors.

In general the results obtained from administration experiments to men and dogs etc. by earlier workers have been repeated in this investigation. No systematic analysis appears to have been carried out in the early experiments, the presence, or absence of acetone, and/or acetoacetic acid, being noted by means of a Rothera test. From these results and the physical symptoms observed, it would/

would appear, however, that the response of the ruminant to the administration of acetone, aceto: acetate and B-hydroxybutyrate is very much the same as that of other species. In all the experimental animals used no clinical symptoms were produced after administration per os, or intravenously, of limited amounts of acetone or B-hydroxybutyric acid, while a strong smell of acetoacetic acid was noted in the breath after administration of that substance. Higher concentrations of acetoacetate produced a state of coma in the rabbit and the cow.

An acetonaemia and acetonuria were observed in rabbits, dogs, cows and sheep after administration per os or intravenously of both acetone and aceto: acetic acid, though when the latter was given intravenously the response was very slight. B-hydroxy: butyric acid administration by either method provoked no response in the subjects used, though Allen and Wishart observed a slight acetonuria inaa rabbit after giving B-hydroxybutyric acid by stomach tube. As iso-propanol is formed in the rumen the response of man and dog etc. to its administration would probably be different from that observed in the ruminant. No references could be found in the literature to investigations concerning the adminis: tration of iso-propanol to any species, so we are unable/

unable to compare the response of the ruminant to that of any other animal.

The above experiments suggest that there is a high degree of interchange between acetone, aceto: acetic acid and iso-propanol in the blood, tissues and rumen, and that B-hydroxybutyric acid is the end point in these conversions, being metabolised directly by the tissues without the production of acetone body intermediates. The site of formation of iso-propanol appears to be the rumen.

SUMMARY.

1. The administration of acetone, aceto: acetic acid and iso-propanol separately to a ruminant, by mouth or intravenous injection, was found to produce a transient form of ketosis.

2. A high degree of interchange was found to exist between these acetone bodies in the blood, tissues and rumen. The latter organ appeared to be the site of formation of iso-propanol.

3. The administration of B-hydroxybutyric acid produced no ketosis, and this fraction appeared to be the end-point of inter acetone body conversions.

4./

4. Acetoacetic acid produced a coma in the cow when present in concentrations above about 25 mg. acetone/100 ml. in the blood.

5. A cycle of events is suggested for the metabolism of the acetone bodies in the blood and tissues, and in the rumen.

6. The results obtained from sheep and cows are compared and discussed in relation to each other and the results obtained from other species.

V. GENERAL DISCUSSION.

DISCUSSION.

The experiments described above have suggested that acetone, acetoacetic acid and B-hydroxybutyric acid are formed in the body, while iso-propanol is formed exogenously in the rumen, possibly from acetone. In no case did the administration of a particular acetone body produce a ketosis, however temporary, completely identical with that produced on starvation. In general the percentage of aceto: acetic acid found in starvation was higher than after administration. Even the injection of acetoacetate itself, by mouth or intravenously, did not produce a similar state; in both cases the very temporary ketosis produced was due initially to a very high percentage of acetoacetic acid, and later of B-hydroxy: butyric acid.

In starvation ketosis the most prominent ex: cretory product, in the cow, was acetoacetic acid, followed by B-hydroxybutyric acid, acetone (in increasing quantities over the period of fast studied), and iso-propanol, in that order. Even immediately after the administration of acetoacetate the pro: portions of this factor and B-hydroxybutyric acid found in the urine were about equal, and subsequent urine samples contained increasing amounts of the latter substance. After the administration of acetone/

acetone and iso-propanol the urine contained B-hydroxybutyric acid in the largest proportions, followed by acetone and iso-propanol respectively, though the iso-propanol was soon superceded by acetone. It would appear that the renal threshold for acetone is low, as it was excreted in large quantities immediately after administration, and about two hours after the administration of iso-propanol. In starvation ketosis, where the level of acetone in the blood is high but about equal to B-hydroxybutyric acid, the main excretory product is acetoacetic acid (about 40% of the total), with, after five days offast, about equal amounts of acetone and B-hydroxybutyric acid (about 25% of the total each). These results suggest that the ketosis observed on fasting a ruminant was not due, initially, to the production, and/or accumulation, of acetone, B-hydroxybutyric acid or iso-propanol in the body tissues or fluids, or in the rumen. But the figures do indicate, particularly those of the cow and sheep in late pregnancy, that the continual excessive production of acetoacetic acid from fat by the liver in starvation, might be the cause. Repeated intra: venous injections of acetoacetate produced a slight ketosis, similar, in the proportions of the various acetone/

acetone bodies, to the starvation ketosis in cows and sheep in late pregnancy.

In both starvation and administration exper: iments to sheep, generally lower percentages of acetone and iso-propanol were found, with higher percentages of acetoacetic acid and B-hydroxybutyric acid than in the cow under the same conditions. This suggests that there is a slight species difference in the metabolism of the ketones. Presuming that acetoacetic acid is formed during the catabolism of fat it appears that both cows and sheep, under normal conditions reduce this substance on formation to B-hydroxybutyric acid. In this form it passes to the tissues, by means of the blood stream, where it is probably metabolised for energy purposes, presumably either directly or after reconversion to acetoacetate. To meet the energy demands of the body during starvation more fat is metabolised, and excessive quantities of acetoacetic acid are formed. The cow decarboxylises a large proportion of the excess to acetone, part of which, after diffusing into the rumen, is converted to iso-propanol. The sheep on the other hand appears t able to tolerate higher concentrations of acetoacetic acid, and reduces a larger proportion of the excess than the cow to B-hydroxybutyric acid, so that less acetone/

acetone, and subsequently less iso-propanol, are found in the blood.

Apart from the slight species difference noted, the ketosis obtained on fasting a cow or a sheep in late pregnancy were very similar and, with regard to the proportions of the various acetone bodies, rather different from that produced on fasting a cow at peak lactation. In general the percentage of B-hydroxybutyric acid was lower, and the percentages of acetone, acetoacetic acid and iso-propanol higher in the cow at peak lactation. Over a five day period of fast the rise in total acetone bodies in the blood of the cow in late pregnancy and one at peak lactation were much the same, though in the former case the level did not increase till the second day of starvation. The difference on fasting cows at peak lactation from those in late pregnancy may be associated with a greater amount of metabolism of fat, in the former case, to such an extent that the normal mechanisms for reducing the acetoacetic acid formed to B-hydroxybutyric acid, are unable to detoxify the former with sufficient speed, so that a larger proportion is decarboxylated to acetone. Alternatively, similar amounts of fat may be catabolised in both cases but, due to some metabolic change associated with parturition or milk production, the cow/

the cow at peak lactation is unable to reduce the excess acetoacetic acid with the same efficiency and decarboxylation takes place instead.

The chain of events suggested by this investigation is primarily the formation of acetoacetate from the catabolism of fat in the liver and its rapid conversion to B-hydroxybutyric acid under normal conditions. Under conditions of stress, such as starvation, the catabolism of fat is increased and the acetoacetic acid formed is rapidly excreted or converted into acetone and B-hydroxybutyric acid. These various fractions gain the blood stream whence they pass into the milk, urine and rumen. In this organ there occurs the formation of iso-propanol, possibly from acetone, which then passes back into the blood stream and eventually reaches the milk and urine though at very low levels. This cycle of events is a dynamic one, and, with the exception of B-hydroxybutyric acid, the acetone bodies are all readily inter-convertible.

VI. SUMMARY.

SUMMARY.

In order to carry out a detailed investigation into the origin and fate of the acetone bodies in the normal ruminant, a method was devised for the estimation of individual acetone bodies and iso-propanol.

A detailed study of the fasting ketosis of both cows and sheep, and the metabolism of the individual ketones and iso-propanol in these two species was carried out. The results obtained indicated that acetone, acetoacetic acid and B-hydroxy: butyric acid originate in the tissues, but the site of origin of iso-propanol is most probably the rumen. A very high degree of interchange was found to occur between acetone, acetoacetic acid and iso-propanol in the blood, tissues, and rumen, but B-hydroxybutyric acid appeared to be the end-point of the conversions, being metabolised by the tissues withouth the pro: duction of the other ketones or iso-propanol.

Conditions of stress, such as fasting, produce a ketosis in both sheep and cows, and though there appears to be a slight species difference in the proportions of the individual ketones formed, the response of the two species in late pregnancy is very similar, and differs from that produced at peak/

peak lactation.

These results are discussed and compared with those obtained by other workers in various animal species.

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VIII. APPENDIX.

TABLES 23 - 38.

TABLE 23.

Recovery of mixtures of Acetone Bodies using standard solutions.

	Substance.	Theoretical Result. mg./100 ml.	Amount Found mg./100 ml.	Error %
1.	Acetone.....	7.92	7.75	- 2.1
	Acetoacetic acid.....	3.81	3.68	- 3.4
	Iso-propanol.....	7.43	7.50	+ 0.9
	B-hydroxybutyric acid.	5.08	5.30	+ 4.3
2.	Acetone.....	7.92	7.75	- 2.1
	Acetoacetic acid.....	3.81	3.81	0
	Iso-propanol.....	7.43	7.15	- 3.7
	B-hydroxybutyric acid.	10.15	9.99	- 1.6
3.	Acetone.....	15.84	15.30	- 3.4
	Acetoacetic acid.....	7.62	7.45	- 2.2
	Iso-propanol.....	14.86	14.00	- 5.7
	B-hydroxybutyric acid.	15.45	14.60	- 5.5

TABLE 24.

Recovery of Mixtures.

	Amount Added mg./100 ml.	Amount Found mg./100 ml.	Blank. mg./100 ml.	Amount Recovered mg./100 ml.	Error %
In Blood.					
1. Acetone.....	7.92	7.87	0	7.87	- 0.6
Acetoacetic acid.....	3.81	3.63	0	3.63	- 5.8
Iso-propanol.....	7.43	7.25	0	7.25	- 2.4
B-hydroxybutyric acid.	5.08	9.60	4.30	5.30	+ 4.3
2. Acetone.....	7.92	8.03	0	8.03	+ 1.0
Acetoacetic acid.....	3.81	3.53	0	3.53	- 7.0
Iso-propanol.....	7.43	7.25	0	7.25	- 2.4
B-hydroxybutyric adid.	10.15	18.90	8.20	10.70	+ 5.4
3. Acetone.....	15.84	15.30	0	15.30	- 3.4
Acetoacetic acid.....	7.62	7.46	0	7.46	- 0.2
Iso-propanol.....	14.86	15.00	0	15.00	+ 0.9
B-hydroxybutyric acid.	15.45	23.17	7.18	15.99	+ 3.5

TABLE 24 (Contd).

Recovery of Mixtures.

	Amount Added mg./100 ml.	Amount Found mg./100 ml.	Blank. mg./100 ml.	Amount Recovered mg./100 ml.	% Error
In Rumen Contents.					
1. Acetone.....	7.92	7.87	0	7.87	- 0.6
Acetoacetic acid.....	3.81	3.93	0	3.93	+ 3.2
Iso-propanol.....	7.43	7.25	0	7.25	- 2.4
B-hydroxybutyric acid.	5.08	8.08	3.17	4.91	- 3.3
2. Acetone.....	15.84	15.50	0	15.50	- 2.1
Acetoacetic acid.....	7.62	7.10	0	7.10	- 6.8
Iso-propanol.....	14.86	14.40	0	14.40	- 3.1
B-hydroxybutyric acid.	15.45	18.50	3.03	15.47	+ 0.1

TABLE 25.

Recovery of mixtures of ketone bodies using standard solutions by Van Slyke's method.

	Theoretical Result mg./100 ml.	Amount Found mg./100 ml.	Error %
1. Acetone and acetoacetic acid.....	11.73	14.00	+ 19.35
B-hydroxybutyric acid and iso-propanol....	12.51	18.48	+ 47.72
Total acetone bodies...	24.24	19.84	- 18.15
2. Acetone and acetoacetic acid.....	11.73	16.60	+ 41.52
B-hydroxybutyric acid and iso-propanol....	17.58	20.59	+ 17.12
Total acetone bodies...	29.31	48.36	+ 64.99
3. Acetone and acetoacetic acid.....	23.46	30.60	+ 30.43
B-hydroxybutyric acid and iso-propanol....	30.31	34.58	+ 14.09
Total acetone bodies...	53.77	62.49	+ 16.22

TABLE 26.

Recovery of Mixtures using Van Slyke's Method.

	Amount Added mg./100 ml.	Amount Found mg./100 ml.	Blank. mg./100 ml.	Amount Recovered mg./100 ml.	% Error
In Blood.					
1. Acetone and acetoacetic acid.....	11.73	5.40	0	5.40	- 53.96
B-hydroxybutyric acid and iso-propanol....	12.51	23.38	1.72	21.66	+ 73.14
Total acetone bodies...	24.24	31.87	1.72	30.15	+ 24.38
2. Acetone and acetoacetic acid.....	11.73	7.30	0	7.30	- 37.77
B-hydroxybutyric acid and iso-propanol....	17.58	27.02	7.99	20.03	+ 13.94
Total acetone bodies...	29.31	30.75	7.99	22.76	- 22.35
3. Acetone and acetoacetic acid.....	23.46	25.30	0	25.30	+ 7.84
B-hydroxybutyric acid and iso-propanol....	30.31	42.28	7.99	34.29	+13.13
Total acetone bodies...	53.77	65.30	7.99	57.31	+ 6.58

TABLE 26 (Contd).

Recovery of Mixtures using Van Slyke's Method.

	Amount Added mg./100 ml.	Amount Found mg./100 ml.	Blank. mg./100 ml.	Amount Recovered mg./100 ml.	% Error
In Rumen Contents.					
1. Acetone and acetoacetic acid.....	11.73	5.20	0	5.20	- 55.67
B-hydroxybutyric acid and iso-propanol....	12.51	19.80	1.96	17.84	+ 43.87
Total acetone bodies...	24.24	26.54	1.98	24.58	+ 1.40
2. Acetone and acetoacetic acid.....	23.46	18.00	0	18.00	- 23.27
B-hydroxybutyric acid and iso-propanol....	30.31	34.58	1.82	32.76	+ 8.08
Total acetone bodies...	53.77	52.34	1.82	50.52	- 6.04

TABLE 27.

Acetone Bodies during fasting a dry cow in late pregnancy.

(As mg. acetone/100 ml.)

Blood.	Days of Fast.					
	0	1	2	3	4	5
Acetone.....	0	0	0.63	1.53	4.57	12.60
Acetoacetic acid.....	0	0	0.11	0.81	3.05	1.78
B-hydroxybutyric acid.	10.67	7.38	14.77	21.50	24.90	30.42
Iso-propanol.....	0	0	0	0.14	0.20	0.58
Total acetone bodies..	10.67	7.38	15.51	23.98	32.72	45.38

TABLE 28.

Average concentrations of acetone bodies
(in mg. acetone/100 ml.)

	Days of Fast.						Mean.
	0	1	2	3	4	5	
Blood.							
Acetone.....	0.03	1.48	4.46	8.96	13.36	15.05	7.22
Acetoacetic acid.....	0.66	1.63	3.04	4.62	5.25	4.39	3.26
B-hydroxybutyric acid..	6.03	8.81	14.54	16.73	12.32	23.46	13.65
Iso-propanol.....	0.00	0.03	0.32	1.56	1.61	1.92	0.91
Total acetone bodies...	6.72	11.94	22.37	31.87	32.55	44.82	25.04
Rumen.							
Acetone.....	0.00	0.88	2.47	5.54	9.68	11.47	5.01
Acetoacetic acid.....	0.22	0.27	1.27	0.83	1.24	1.65	0.91
B-hydroxybutyric acid..	3.69	5.52	4.88	8.52	6.39	3.96	5.49
Iso-propanol.....	0.00	0.47	1.08	3.33	5.22	5.47	2.59
Total acetone bodies...	3.91	7.14	9.70	18.22	22.53	22.55	14.00
Milk.							
Acetone.....	0.00	1.39	3.60	8.69	14.50	16.24	7.40
Acetoacetic acid.....	0.00	0.27	2.67	0.84	1.79	1.57	1.19
B-hydroxybutyric acid..	4.92	5.35	10.79	16.00	8.53	7.92	8.92
Iso-propanol.....	0.00	0.05	0.15	1.10	1.44	1.77	0.75
Total acetone bodies...	4.92	7.06	17.21	26.63	26.26	27.50	18.26
Urine.							
Acetone.....	1.01	6.08	8.93	16.03	25.04	21.99	13.18
Acetoacetic acid.....	3.37	31.32	31.09	80.36	40.89	37.29	37.39
B-hydroxybutyric acid..	11.66	19.01	8.61	53.05	35.38	25.10	25.47
Iso-propanol.....	0	0.15	0.49	12.09	2.90	4.63	3.37
Total acetone bodies...	16.04	56.56	49.12	161.53	104.21	89.01	79.41

TABLE 29.

Acetone Bodies of a cow
with subclinical bovine ketosis during fasting.

(As percentage of total)

	Days of Fast.						
	-1	0	1	2	3	4	5
1. Blood.							
Acetone.....	30.68	21.41	37.91	31.32	39.95	44.44	58.62
Acetoacetic acid.....	12.76	15.31	9.19	26.94	9.68	12.17	14.62
B-hydroxybutyric acid.	55.34	62.31	50.19	37.83	39.07	25.65	17.15
Iso-propanol.....	1.22	0.97	2.71	3.91	11.30	19.74	9.61
2. Rumen Contents.							
Acetone.....	31.20	40.21	59.20	44.99	60.71	71.75	76.03
Acetoacetic acid.....	4.55	3.04	4.36	11.92	4.66	12.58	7.27
B-hydroxybutyric acid.	16.87	21.57	12.62	8.45	11.13	0.43	3.51
Iso-propanol.....	47.38	35.18	23.82	34.64	23.50	15.24	13.19

TABLE 30.

Concentrations of acetone bodies on fasting.

(In mg. acetone/100 ml.).

	Days of Fast.				
	0	1	2	3	4
A. Non-pregnant ewe.					
Acetone.....	0	0	0	0	0.81
Acetoacetic acid.....	0	0	0	1.23	0.91
B-hydroxybutyric acid.	11.68	10.05	16.82	10.87	20.28
Iso-propanol.....	0	0	0	0	0.26
Total acetone bodies..	11.68	10.05	16.82	12.10	22.26
B. Pregnant ewe.					
Acetone.....	8.10	8.10	10.44	10.98	12.60
Acetoacetic acid.....	5.43	18.35	9.86	6.86	9.93
B-hydroxybutyric acid.	16.07	12.52	39.20	23.17	54.42
Iso-propanol.....	0.20	1.10	0.83	0.24	0.56
Total acetone bodies..	29.80	39.07	60.33	41.25	76.51
C. Pregnant ewe.					
Acetone.....	0.63	0.81*	0.54		
Acetoacetic acid.....	0.11	1.53	1.55		
B-hydroxybutyric acid.	12.92	22.75	12.25		
Iso-propanol.....	0	0	0		
Total acetone bodies..	13.66	25.09	14.34		

* Ewe lambed between 1st and 2nd days of fast.

TABLE 31.

Acetone Bodies after administration of acetone into the rumen.

A. - 100 ml. to a cow.
B. - 4 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
A. 100 ml. to a cow.				
1. Blood.				
Acetone.....	0	4.05	13.50	14.95
Acetoacetic acid.....	0	1.00	1.26	0.43
B-hydroxybutyric acid.	4.52	16.18	1.52	8.55
Iso-propanol.....	0	0	1.55	2.25
2. Rumen Contents.				
Acetone.....	0	67.50	21.60	15.75
Acetoacetic acid.....	0	0.77	1.16	0.61
B-hydroxybutyric acid.	2.87	2.93	1.00	1.80
Iso-propanol.....	0	13.00	16.00	17.00
3. Milk.				
Acetone.....	0	1.13	12.38	6.08
Acetoacetic acid.....	0	1.95	3.92	6.53
B-hydroxybutyric acid.	4.62	30.75	16.75	7.20
Iso-propanol.....	0	0	0.90	0.60

TABLE 31 (Contd).

Acetone Bodies after administration of acetone into the rumen.

A. - 100 ml. to a cow.
B. - 4 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
B. 4 ml. to a sheep.				
1. Blood.				
Acetone	0	0.54	1.80	3.78
Acetoacetic acid	0	0.24	0.66	1.88
B-hydroxybutyric acid.	12.30	13.12	11.93	13.78
Iso-propanol	0	0	0.22	0.22
2. Rumen Contents.				
Acetone	0	30.60	6.84	7.38
Acetoacetic acid	0	0.15	2.51	2.62
B-hydroxybutyric acid.	7.12	1.42	4.17	3.17
Iso-propanol	0	1.00	2.30	2.10

TABLE 32.

Acetone Bodies after administration of acetone intravenously

A. - 75 ml. to a cow.
B. - 4 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
A. 75 ml. to a cow.				
1. Blood.				
Acetone.....	0	7.83	5.85	4.68
Acetoacetic acid.....	0	0.41	0.12	0.36
B-hydroxybutyric acid.	2.87	9.38	11.25	11.48
Iso-propanol.....	0	0.28	0.20	0
2. Rumen Contents.				
Acetone.....	0	1.26	1.71	1.80
Acetoacetic acid.....	0	0.09	0.50	0.17
B-hydroxybutyric acid.	1.65	10.25	4.68	2.52
Iso-propanol.....	0	1.11	3.60	5.50
3. Milk.				
Acetone.....	0	1.35	5.63	2.70
Acetoacetic acid.....	0	1.73	0.52	3.14
B-hydroxybutyric acid.	4.10	7.17	6.15	8.20
Iso-propanol.....	0	0	0	0

TABLE 32 (Contd).

Acetone Bodies after administration of acetone intravenously

A. - 75 ml. to a cow.
B. - 4 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
B. 4 ml. to a sheep.				
1. Blood.				
Acetone.....	0	19.13	20.25	6.75
Acetoacetic acid.....	0	0.86	1.28	1.25
B-hydroxybutyric acid.	0	22.45	21.22	5.27
Iso-propanol.....	0	0.36	1.10	2.67
2. Rumen Contents.				
Acetone.....	0	0	9.00	0.56
Acetoacetic acid.....	0	0	3.30	2.52
B-hydroxybutyric acid.	0	12.68	12.72	1.80
Iso-propanol.....	0	3.15	7.75	8.75

TABLE 33.

Acetone Bodies after administration of ethyl acetoacetate into the rumen

A. - 150 ml. to a cow.
B. - 4 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
A. 150 ml. to a cow.				
1. Blood.				
Acetone.....	0	0	0.36	0.18
Acetoacetic acid.....	0	2.95	0.99	0.31
B-hydroxybutyric acid.	3.08	24.82	22.35	20.72
Iso-propanol.....	0	0	0	0
2. Rumen Contents.				
Acetone.....	0	0.54	0.36	0
Acetoacetic acid.....	0	22.83	5.18	0.25
B-hydroxybutyric acid.	2.87	1.22	7.20	13.43
Iso-propanol.....	0	0.50	0.60	0.30
3. Milk.				
Acetone.....	0	0	0	0
Acetoacetic acid.....	0	2.77	0.93	0.93
B-hydroxybutyric acid.	4.62	7.88	5.13	10.75
Iso-propanol.....	0	0	0	0

TABLE 33 (Contd).

Acetone Bodies after administration of ethyl acetoacetate into the rumen

A. - 150 ml. to a cow.
B. - 4 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
B. 4 ml. to a sheep.				
1. Blood.				
Acetone.....	0	0	1.69	0
Acetoacetic acid.....	0	0	4.46	0
B-hydroxybutyric acid.	0	10.15	10.25	6.15
Iso-propanol.....	0	0	0	0
2. Rumen Contents.				
Acetone.....	0	13.50	1.69	0
Acetoacetic acid.....	0	87.10	25.99	0
B-hydroxybutyric acid.	0	0.15	0.53	5.42
Iso-propanol.....	0	0.45	2.13	1.05

TABLE 34.

Acetone Bodies after administration of iso-propanol into the rumen

A. - 250 ml. to a cow.
B. - 15 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
A. 250 ml. to a cow.				
1. Blood.				
Acetone.....	0	4.77	15.30	23.85
Acetoacetic acid.....	0	0.40	1.31	5.67
B-hydroxybutyric acid.	3.48	0.77	18.93	4.68
Iso-propanol.....	0	15.90	20.00	13.80
2. Rumen Contents.				
Acetone.....	0	5.40	16.65	22.50
Acetoacetic acid.....	0	3.58	0.57	0.87
B-hydroxybutyric acid.	2.25	43.02	0.18	3.53
Iso-propanol.....	0	247.50	62.00	52.00
3. Milk.				
Acetone.....	0	0	12.15	20.25
Acetoacetic acid.....	0	0	0.77	2.51
B-hydroxybutyric acid.	8.02	29.22	43.53	19.67
Iso-propanol.....	0	4.00	20.00	18.00

TABLE 34 (Contd).

Acetone Bodies after administration of iso-propanol into the rumen

A. - 250 ml. to a cow.
B. - 15 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
B. 15 ml. to a sheep.				
1. Blood.				
Acetone.....	0	2.79	6.84	8.64
Acetoacetic acid.....	0	0.90	1.40	1.49
B-hydroxybutyric acid.	12.88	0.52	7.68	12.12
Iso-propanol.....	0	3.75	2.53	1.05
2. Rumen Contents.				
Acetone.....	0	0.81	3.60	6.38
Acetoacetic acid.....	0	0.30	0.83	1.24
B-hydroxybutyric acid.	10.25	0.52	9.47	10.95
Iso-propanol.....	0	65.00	68.00	43.00

TABLE 35 (Contd).

Acetone Bodies after administration of iso-propanol intravenously

A. - 125 ml. to a cow.
B. - 4 ml. to a sheep.

(As mg. acetone/100 ml.)

	Time after administration.			
	0	10 mins.	1 hr.	2 hrs.
B. 4 ml. to a sheep.				
1. Blood.				
Acetone.....	0	0	15.78	13.05
Acetoacetic acid.....	0	0	1.16	2.33
B-hydroxybutyric acid.	5.13	14.78	2.12	0.35
Iso-propanol.....	0	29.59	9.50	4.40
2. Rumen Contents.				
Acetone.....	0	0	1.13	5.63
Acetoacetic acid.....	0	0	1.95	0.52
B-hydroxybutyric acid.	2.57	6.37	0	0.84
Iso-propanol.....	0	3.25	9.22	9.00

TABLE 36.

Acetone Bodies after administration of acetone intravenously four times daily to a sheep.

(As percentage of total).

	Days of Experiment.					
	0	1	2	3	4	5
Acetone injected.	4 x 2.5ml.	4 x 2.5ml.	4 x 5ml.	4 x 5ml.		
1. Blood.						
Acetone.....	0	47.46	59.91	76.53	66.01	15.65
Acetoacetic acid.....	0	28.00	15.47	12.48	10.33	5.74
B-hydroxybutyric acid	100	20.30	20.46	5.20	20.10	78.61
Iso-propanol.....	0	4.24	4.16	5.79	3.56	0
Total acetone bodies (mg. acetone/100 ml.).	7.78	16.50	18.75	27.63	35.45	5.75
2. Rumen Contents.						
Acetone.....	0	42.11	63.33	63.30	71.08	8.91
Acetoacetic acid.....	0	7.32	9.40	4.22	11.59	3.27
B-hydroxybutyric acid	100	28.67	7.57	17.99	7.08	73.56
Iso-propanol.....	0	21.90	19.70	14.58	10.25	14.26
Total acetone bodies (mg. acetone/100 ml.).	2.47	16.67	17.75	29.15	29.75	10.10

TABLE 37.

Acetone Bodies after administration of iso-propanol into the rumen of a sheep four times daily.

(As percentage of total)

	Days of Experiment.					
	0	1	2	3	4	5
Iso-propanol injected.	4 x 5ml.	4 x 5ml.	4 x 5ml.	4 x 10ml.		
1. Blood.						
Acetone.....	16.38	29.46	28.25	25.74	63.77	0
Acetoacetic acid.....	1.52	1.17	6.61	2.68	14.21	0
B-hydroxybutyric acid.	72.10	68.48	60.08	69.56	18.37	100
Iso-propanol.....	0	0.89	5.06	2.02	3.65	0
Total acetone bodies (mg. acetone/100 ml.)..	16.48	28.11	29.63	27.27	26.82	10.45
2. Rumen Contents.						
Acetone.....	0	46.10	58.75	23.78	55.56	0
Acetoacetic acid.....	0	1.17	2.81	2.31	8.01	0
B-hydroxybutyric acid	100	36.03	16.59	57.54	24.80	100
Iso-propanol.....	0	16.70	21.85	16.37	11.63	0
Total acetone bodies (mg. acetone/100 ml.)..	10.25	17.96	16.24	20.77	34.82	1.85

TABLE 37a (Contd).

Acetone Bodies after administration of iso-propanol into the rumen of a sheep four times daily.

(As percentage of total)

	Days of Experiment.											
	0	1	2	3	4	5	6	7	8	9	10	11
Iso-propanol injected.	4 x 4 ml.	4 x 2 ml.	4 x 1 ml.	4 x 2 ml.	4 x 2 ml.	2 x 6 ml.	1 x 20ml.	4 x 4 ml.	4 x 5 ml.			
2. Rumen Contents.												
Acetone.....	0									38.13	46.44	20.53
Acetoacetic acid.....	0									6.54	1.16	3.87
B-hydroxybutyric acid.	100									17.51	17.03	6.20
Iso-propanol.....	0									37.82	35.47	69.40
Total Acetone Bodies (mg. acetone/100 ml.)	9.43									66.10	31.01	10.09

TABLE 38.

Acetone Bodies after administration of 30 ml. ethyl acetoacetate intravenously to a cow four times daily.

1. As percentage of total.

	Blood.	Rumen Contents.	Milk.	Urine.
A. 2.15 p.m.				
Acetone	6.45	5.82	0	8.79
Acetoacetic acid	2.37	3.45	11.09	16.56
B-hydroxybutyric acid.	91.18	84.26	88.91	74.65
Iso-propanol	0	6.47	0	0
B. 4.45 p.m.				
Acetone	9.67	13.43	16.66	16.16
Acetoacetic acid	5.38	8.06	6.17	24.49
B-hydroxybutyric acid.	83.66	66.58	69.76	53.19
Iso-propanol	1.29	11.93	7.41	6.25

2. As mg. acetone/100 ml.

A. 2.15 p.m.				
Acetone	0.90	0.54	0	1.62
Acetoacetic acid	0.33	0.32	1.85	3.05
B-hydroxybutyric acid.	12.72	7.82	14.85	13.75
Iso-propanol	0	0.60	0	0
B. 4.45 p.m.				
Acetone	0.90	0.45	1.35	2.07
Acetoacetic acid	0.50	0.27	0.50	3.14
B-hydroxybutyric acid.	7.70	2.23	5.65	6.80
Iso-propanol	0.12	0.40	0.60	0.80

TABLE 37a.

Acetone Bodies after administration of iso-propanol into the rumen of a sheep four times daily.

(As percentage of total)

	Days of Experiment.											
	0	1	2	3	4	5	6	7	8	9	10	11
Iso-propanol injected.	4 x 4 ml.	4 x 2 ml.	4 x 1 ml.	4 x 2 ml.	4 x 2 ml.	2 x 6 ml.	1 x 20ml.	4 x 4 ml.	4 x 5 ml.			
1. Blood.												
Acetone.....	0	68.73	51.27	44.52	70.98			58.40	68.88	59.58	58.54	39.79
Acetoacetic acid.....	0	3.14	10.55	3.29	14.20			3.61	1.72	14.23	11.46	9.07
B-hydroxybutyric acid.	100	23.33	23.30	43.53	1.55			17.49	1.54	2.19	20.78	48.29
Iso-propanol.....	0	4.80	14.88	8.66	13.27			20.50	27.86	24.00	9.22	2.25
Total acetone bodies. (mg. acetonw/100 ml.)...	7.78	18.33	29.84	28.30	17.33			59.50	47.03	58.32	36.90	13.34