STUDIES ON KETOSIS IN DAIRY CATTLE. XI. LIPIDS, MINERALS AND ASCORBIC ACID IN THE BLOOD OF COWS WITH SPONTANEOUS KETOSIS[1]

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Rather extensive fatty infiltration and degeneration of various organs of cows with ketosis have been observed in studies carried on in this laboratory (10). This suggested that disturbances in fat metabolism may be involved. It appeared advisable, therefore, to determine whether any consistent abnormalities existed in the various blood lipids of cows with ketosis, since little information of this nature was available.

The adrenal cortex of ketotic cows always has shown extensive degeneration (11). Prior to these studies it was shown (9) that an extract of the adrenal cortex was effective in the treatment of ketosis. To obtain additional information on the possible role of the adrenals in the development of ketosis, an investigation of the level of ascorbic acid, sodium and potassium in the blood plasma also was included in this study. In addition, blood chlorides, phosphates and phosphatase activity were determined.

EXPERIMENTAL PROCEDURE

All of the cases reported herein were field cases diagnosed as ketosis by practicing veterinarians. One of the cows studied had had ketosis in previous years and was subjected to study before and during the development of the ketotic condition. In all cases blood glucose and acetone bodies were determined as an aid in diagnosis. Some of these cases were used simultaneously for other investigations which will be reported later. The methods used are as follows: Glucose, Somogyi's (13) modification of the Shaffer and Somogyi procedure used on cadmium sulphate filtrates; acetone bodies, Barnes and Wick (1); ascorbic acid, Mindlin and Butler (5); sodium, a modification of Snell and Snell (12); potassium, Harris (2); chlorides, Whitehorn (14); phosphates, Saarinen's (6) modification of the Kuttner, Cohen and Lichtenstein procedure. This same procedure also was used in determining phosphatase activity.

The method used for blood lipids is a modification of one developed by one of the authors (7, 8). A modification of Bloor's method, developed by Katsura and associates (3, 4), was used for a comparison in developing the shorter method used in this study.

In this study instead of separating the phospholipids by precipitation, they were calculated from the difference between the amount of total lipids and the other lipids that were extracted separately. This separation is based upon the heavy hydration of phospholipids in certain pH areas where phospholipids are not extractable from aqueous emulsion with ether and petroleum ether.

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For extraction the plasma pH is changed with a buffer solution, which liberates lipids from lipoprotein combinations so that all lipids except the phospholipids are extractable as such with low-boiling dry solvents. One method for complete fractionation of the plasma lipids based upon this procedure was published earlier by Saarinen (6, 7). In the present study instead of using this more accurate alcohol ether extraction, the total lipids were extracted from an alkaline aqueous emulsion with dry solvents after dehydrating the phospholipids with alcohol. This procedure is described in some detail.

The extraction of the total lipids (A). Measure 2 ml. of mixed plasma into a 25-ml. glass-stoppered graduate cylinder. Wash the pipette into the cylinder with 2 ml. of distilled water. Add 0.25 ml. of concentrated ammonium hydroxide. After mixing, add 2 ml. of redistilled alcohol (95 per cent) and mix again. Add 10 ml. of redistilled ethyl ether. Tighten the stopper with distilled water and shake vigorously by hand for 1 min. Loosen the stopper to release the pressure and allow to stand approximately 30 min. Repeat the shaking four times, allowing the mixture to stand 5 min. between shakings. Then add 10 ml. of petroleum ether and repeat the shaking four times as above. Wash the stopper with distilled water and let the cylinder stand over night or for at least 6 hr. The extract, in which 10 ml. are equivalent to 1 ml. of plasma, contains all the plasma lipids except free fatty acids but these normally are present only in negligible amounts.

The extraction of plasma lipids other than phospholipids (B). Measure 4 ml. of plasma into a 50-ml. glass-stoppered graduate cylinder and wash the pipette into the cylinder with 4 ml. of distilled water. Add 0.80 ml. of acetate buffer stock solution (one volume 1 *N* sodium hydroxide plus two volumes of 1 *N* acetic acid), followed by 20 ml. of redistilled ethyl ether. Stopper with moistened glass stopper and shake carefully for 30 sec. Loosen the stopper to release the pressure, stopper again and shake vigorously for 2 min. Then let stand for 20 to 30 min. After standing, shake four times, shaking for 2 min. each time and allowing to stand for 5 min. between shakings. After this extraction, 20 ml. of petroleum ether is added and the shaking is repeated four times, shaking for 1 min. each time and allowing to stand for 5 min. between shakings.

This extract, in which 10 ml. is equivalent to 1 ml. of plasma, contains all of the plasma lipids except the phospholipids.

The total amount of lipids in each extract is determined oxidometrically using the technique of Katsura *et al.*, modified as explained later. The same technique also is used for precipitating free cholesterol. The cholesterol determinations, however, are made colorimetrically, using a modification of Urbach as given by Zeiss (15).

Oxidometric determination of lipids in extracts A and B. Duplicate 5 ml. quantities of each extract (equivalent to 0.5 ml. plasma) are measured into acid-washed Erlenmeyer flasks of 50-ml. volume and evaporated to dryness over a boiling water bath. Traces of ammonia and acetic acid are driven off by blowing air into the flasks. Then the flasks are placed in a drying oven at

100° C. for 10 min. and air again is blown through the flasks several times. After cooling, 3 ml. of an oxidation mixture consisting of four parts of Nicloux silver dichromate-sulfuric acid solution and one part of 1 *N* aqueous potassium dichromate solution are added to each flask by means of an Ostwald pipette with a sharp tip. Two blank determinations are made simultaneously using the same procedure. The flasks are stoppered and placed over a boiling water bath for exactly 30 min. During this time, the flasks are rotated gently until all of the lipid material is dissolved, as will be indicated by a uniform adherence of the oxidation mixture on the surface of the flask. The flasks next are placed in an oven at 100 to 101° C. for exactly 1 hr., being rotated gently at 15-min. intervals and allowed to cool. After cooling, 25 ml. of distilled water are added to each flask. Just before titration, 2 ml. of 40 per cent slightly alkaline potassium iodide solution are added. The liberated iodine is titrated with a 0.05 *N* sodium thiosulfate solution, using soluble starch (1 per cent in saturated KCl solution) as indicator. The blank minus the actual titration value reveals the amount of chromic acid required for the oxidation of the lipids in extracts A and B.

Determination of total cholesterol. For the determination of total cholesterol, 5 ml. of each extract (A and B) are used for duplicates. If the extractions are carried out properly, the cholesterol will be extracted quantitatively in both cases. Thus, the cholesterol determination also is used to check on the quantitativeness of the extractions, particularly for extraction B which requires greater care.

Five ml. of each extract is measured into a 50-ml. glass-stoppered Erlenmeyer flask, evaporated over a steam bath and dried in an oven as described previously. After cooling, 5 ml. of chloroform and 1 ml. of acetic anhydride are added and mixed. Just before warming, 1 ml. of a solution consisting of nine volumes of acetic anhydride and one volume of concentrated sulfuric acid is added and mixed by rotating. The flasks immediately are placed in the dark in a covered water bath at 38° C. for exactly 15 min. The flasks are cooled and readings are made with a suitable photometer at a wave length of 660 mμ. For calculation a standard curve is established with pure cholesterol. The results are calculated on the assumption that 90 per cent of the value for blood cholesterol is due to true cholesterol (7, pp. 41 and 125).

Ester cholesterol. For each duplicate, 5 ml. of extract B are placed into a 50-ml. Erlenmeyer flask and evaporated to dryness over a water bath and the acetic acid is removed by passing a stream of air into the flask. The residue is redissolved with 5 ml. of acetone, followed by the addition of 2.5 ml. of 0.2 per cent digitonin solution in 95 per cent alcohol and the addition of 0.5 ml. of distilled water. After mixing, the flasks are placed on a water bath in contact with steam. The temperature of the water bath is maintained at 50 to 60° C. for 15 min., after which it gradually is increased to boiling. After evaporation the residue is dried by passing a stream of air slowly through the flask. The cholesterol esters are extracted with ether in a warm flask using three repeated extractions and 3 to 4 ml. of ether for each extraction. The portions are fil-

tered into another glass-stoppered Erlenmeyer flask through a fat-free filter and then evaporated to dryness. After cooling, 5 ml. of chloroform and 1 ml. of acetic anhydride are added and mixed. The remainder of the procedure is the same as for total cholesterol. When a limited number of determinations are being made, time may be saved by extracting the cholesterol esters from the dried residue directly with chloroform.

Acidometric titration of non-volatile free acids in ether-petroleum ether extract. Twenty ml. of extract B are measured into a 50-ml. Erlenmeyer flask for each duplicate and evaporated to dryness as before when the samples are prepared for oxidation of the lipids. This removes the acetic acid present in the extract. After cooling, 10 ml. of a benzene-alcohol mixture (1:1) containing 0.02 per cent phenolphthalein are added and the free acids are titrated with freshly diluted carbonate free 0.01 *N* potassium hydroxide solution using a microburette with 0.01 ml. divisions.

Calculations. Both total and ester cholesterol are calculated using a standard factor and the extinction value (L = 2-log G) as a basis. When the amount needed for complete oxidation of total cholesterol in plasma (3.92 ml. of 0.1 *N* oxidant per 1 mg. of cholesterol) is subtracted from the titration value B (blank minus titration), the difference reveals the amount of oxidant used by the fatty acids in cholesterol and glycerol esters. This divided by the reduction constant of blood fatty acids (3.60 ml. of 0.1 *N* oxidant per 1 mg. of fatty acids) gives the amount of fatty acids in milligrams. The difference between titration values A and B shows the amount used by phospholipids. This value divided by the constant 2.82 gives the amount of phospholipids in milligrams.

If 5 ml. of extract (equivalent to 0.5 ml. plasma) are used for every determination and titrations are made with 0.05 *N* thiosulfate, the calculations of the lipid fractions would be as follows:

(a) Total cholesterol in mg. per cent determined colorimetrically,
(b) Ester cholesterol in mg. per cent determined colorimetrically,
(c) Phospholipids in mg. per cent. = $(A-B)/2.82 \times 100$,
(d) Fatty acids in cholesterol and glycerol esters and as free-fatty acids in mg. per cent = $(B-3.92a)/3.60 \times 100$,
(e) Cholesterol ester fatty acids in mg. per cent = $0.646b$,
(f) Cholesterol esters in mg. per cent = $1.62b$ or $0.972(b+e)$,
(g) Glycerol esters + free fatty acids in mg. per cent = $d-e$,
(h) Total lipids in mg. per cent = $a+c+d$.

RESULTS

Blood plasma lipids. The blood plasma lipid values of cows with ketosis are presented in table 1. Because a number of the cows exhibited complications of various kinds in addition to the ketosis, an attempt was made to group the complicated and uncomplicated cases separately. On the basis of the present knowledge of ketosis in cows, such a differentiation appears advisable. Histopathological studies of cows with ketosis revealed that many cases exhibit some kind of complication other than that which is secondary to the ketotic condition.

TABLE 1

Blood plasma lipids of ketotic cows

Date	Cow	Blood glucose	Blood acetone bodies	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in		Free acids	Days with ketosis and comments
									Choles-terol esters	Glycerol esters and as free		
		(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*m. equiv./* 100 *ml.*)	
					A. Apparently uncomplicated ketosis							
3/24/48	Hall[a]	56.2	10.0	.....	.....	62.3	52.4	9.4	34.1	12.6	.....	20 d., glucose adm.
3/25/48	Hall	37.5	10.9	.....	.....	66.6	48.2	18.4	31.3	6.0	.....	21 d.
3/24/48	Downs I	41.9	13.4	.....	.....	110.9	90.5	20.4	58.8	21.2	.....	7 d., recovering
3/24/48	Downs II	35.1	.....	.....	.....	105.1	82.6	22.5	53.7	6.3	.....	7 d., recovering
3/25/48	Flegel	39.4	20.5	.....	.....	98.2	78.7	19.5	51.2	3.8	.....	6 d., recovering
7/ 9/48	Hermosa[a]	53.1	3.4	269.5	92.9	101.6	97.4	4.2	63.3	11.7	0.08	Prepartum
7/13/48	Hermosa[a]	50.3	5.4	226.0	80.1	80.9	67.7	13.2	43.7	21.3	0.10	Day of parturition
7/22/48	Hermosa[a]	40.4	6.4	238.1	100.0	85.0	56.8	28.2	36.1	17.0	0.11	Postpartum
7/28/A.M.	Hermosa[a]	33.3	14.4	283.2	109.2	116.2	90.5	25.7	57.8	0.0	0.09	1 d., early ketosis
P.M.	Hermosa	23.8	15.5	254.8	79.4	117.1	86.9	30.2	56.4	1.9	0.09	1 d., early ketosis
8/ 2/48	Hermosa[a]	41.7	2.5	286.3	120.6	109.0	103.0	6.0	56.7	0.0	0.11	Recovering
8/ 6/48	Hermosa[a]	38.1	6.0	352.3	150.4	129.1	95.5	33.6	62.0	10.8	0.15	Recovering
7/28/48	Inez	20.5	31.1	245.8	66.0	118.8	101.1	17.7	61.0	0.0	0.10	4 d., glucose adm.
8/ 2/48	Inez[a]	36.7	3.3	233.4	76.6	96.7	80.4	16.3	52.2	7.8	0.12	Recovering, glucose adm.
8/ 6/48	Inez[a]	34.2	5.2	372.7	175.2	125.3	101.3	24.0	65.8	6.4	0.12	Recovering, glucose adm.
8/16/48	Beltsville	22.3	45.0	291.9	104.3	137.6	103.7	33.9	50.0	0.0	0.07	1 d.
8/21/48	Beltsville[a]	31.6	26.8	271.5	96.5	119.5	96.2	23.3	55.5	0.0	0.05	5 d.
7/ 8/49	King	21.6	15.0	340.7	146.1	99.6	65.2	34.4	42.3	52.7	0.19	10 d., responded to glucose adm.
Av. during ketosis		.....	.....	283.3	99.0	106.7	82.1	24.6	50.6	21.0	0.11	

TABLE 1 (*Continued*)

Date	Cow	Blood glucose	Blood acetone bodies	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Choles-terol esters	Glycerol esters and as free	Free acids	Days with ketosis and comments
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*m. equiv./ 100 ml.*)	
						B. Complicated ketosis						
3/ 3/48	Hoffman	18.9	40.2	.....	.....	66.3	52.0	14.3	41.3	42.9	.....	15 d., ruptured hypophysis
3/ 5/48	Hoffman[a]	28.9	30.9	.....	.....	72.3	55.3	17.0	45.9	44.4	.....	17 d.
3/ 3/48	Thoma[a]	23.5	33.7	.....	.....	.....	.....	.....	.....	.....	.....	9 d., glucose adm.
3/ 9/48	Thom	56.2	8.3	.....	.....	61.4	47.2	14.2	30.7	41.0	.....	15 d., pneumonia
3/30/48	Cunningham	24.4	25.1	.....	.....	143.5	111.6	31.9	72.5	20.3	.....	2 d., uterus inflamed
4/ 6/48	Burdette[a]	39.4	5.0	.....	.....	82.6	66.6	16.0	43.6	33.3	0.30	11 d., glucose administration
4/ 7/48	Burdette	41.2	8.1	.....	.....	76.6	63.7	12.9	41.4	35.8	0.28	12 d., atrophied hypophysis
2/ 7/49	Mullinix	33.7	38.9	135.5	22.7	66.1	41.0	25.1	26.7	20.0	0.21	13 d., ilium inflamed, ulcerated
3/16/49	Thomas	19.3	56.8	270.6	92.2	91.7	74.4	17.3	48.4	38.3	0.13	4 d., abomasum & duodenum slightly inflamed
3/30/49	Enterprise	22.1	46.2	314.5	92.9	125.5	102.2	23.3	66.4	29.7	0.20	16 d., excess glucose pumped in rumen
4/28/49	Sherman[a]	17.8	12.8	281.3	130.5	86.9	75.4	11.5	49.0	14.9	0.13	4 d., severe inflammation of abomasum & intestines
5/ 3/49	Sherman	22.9	24.0	307.6	125.3	96.7	74.9	21.8	48.6	37.0	0.18	
Av. during ketosis		.....	.....	257.1	83.3	91.0	70.9	20.1	47.0	33.1	0.20	

[a] Not included in average.

TABLE 2

Blood plasma lipids of cows in early and late stages of ketosis

Date	Cow	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in			Free acids
							Choles-terol esters	Glycerol esters and as free	Total	
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*m. equiv./ 100 ml.*)
				A. Early stage of ketosis (1–4 d.)						
7/28/48	Inez	245.8	66.0	118.8	101.1	17.7	61.0	0.0	61.0	0.10
7/28/48	Hermosa	269.0	94.3	116.7	88.5	28.0	57.1	1.0	58.0	0.09
8/16/48	Beltsville 382	291.9	104.3	137.6	103.7	33.9	50.0	0.0	50.0	0.07
3/16/48	Thomas	270.6	92.2	91.7	74.4	17.3	48.4	38.3	87.7	0.13
	Av.	269.3	89.2	116.2	91.9	24.2	54.1	9.9	64.0	0.10
				B. Medium stage of ketosis (6–7 d.)						
3/24/48	Downs I	.....	.....	110.9	90.5	20.4	58.3	21.2	79.5	
3/24/48	Downs II	.....	.....	105.1	82.6	22.5	53.7	6.3	60.0	
3/25/48	Flegel	.....	.....	98.2	78.7	19.5	51.2	3.8	55.0	
	Av.			104.7	83.9	20.8	54.4	10.4	64.8	
				C. Late stage of ketosis (10–21 d.)						
3/25/48	Hall	.....	.....	66.6	48.2	18.4	31.3	6.0	36.3	.....
7/ 8/49	King	340.7	146.1	99.6	65.2	34.4	42.3	52.7	95.0	0.19
3/ 3/48	Hoffman	.....	.....	66.3	52.0	14.3	41.3	42.9	84.2	.....
2/ 7/49	Mullinix	135.5	22.7	66.1	41.0	25.1	26.7	20.0	46.7	0.21
	Av.	238.1	84.4	74.7	51.6	23.1	35.4	30.4	65.6	0.20

Several cases listed as complicated would have been classified as uncomplicated if the animals had not been slaughtered. It appears, therefore, that at least some of the cows listed here in the uncomplicated group probably represent complicated cases. For example, the cow Hermosa, which appeared to be an excellent example of an early case of spontaneous ketosis, exhibited lipofibroma when posted 1 yr. later. This cow had exhibited ketosis postpartum for 3 consecutive yr. and for this reason the blood picture was followed closely immediately prepartum and postpartum. As will be observed from the blood glucose and acetone body values, blood samples were drawn from this cow before and during the development of ketosis and after recovery. In some of the cases, the blood samples were drawn after the animals had been treated with glucose so that the previous diagnosis of the veterinarian had to be used. Consequently, the blood sugar was higher and the acetone bodies lower than would have been the case before treatment. A number of these cows were slaughtered for more extensive biochemical and histo-pathological studies. Complications frequently were observed in the postmortem examinations. Blood samples frequently were drawn from the same cow in different stages of ketosis.

In computing the averages shown in table 1, the data from a single sample of blood drawn during the height of ketosis was used. Considerable variation was observed in the lipid values. All of the lipid values except the free fatty acid equivalent appeared to be somewhat lower than was to be expected. The free fatty acid equivalent was proportionally high in both complicated and uncomplicated ketosis but was observed to be highest in the cows with complicated ketosis.

These differences appeared to be due to the stage of ketosis, since the complicated cases did not respond to treatment readily and, therefore, often represented later stages of this condition. To determine whether the differences were due primarily to the stage of ketosis, some of the animals with either no complications or less severe complications were grouped into early, medium and late stages of ketosis. None of the cows grouped as early cases had exhibited signs or symptoms of ketosis for more than 4 days. Those classified as medium and late stages had exhibited signs and/or symptoms for 6 to 7 days and 10 to 21 days, respectively. As will be noted in table 2, most of the lipid fractions showed a decrease when the animals exhibited ketosis over a longer period of time. However, the free cholesterol did not appear to change appreciably, whereas the neutral fat and free fatty acid fractions usually increased.

Blood minerals, ascorbic acid and hematocrit values. The data on these substances are presented in table 3 for cows which exhibited ketosis of either a complicated or uncomplicated nature. The inorganic phosphorus varied widely with some values below normal. The values for blood plasma sodium, potassium and chlorine were within the normal range in most cases. The plasma ascorbic acid values also varied widely. Some of the values were low, but since the majority of the values were within the normal range it does not appear that there is any specific relationship between the blood plasma ascorbic acid and

ketosis in cows. The red cell volume usually was high, undoubtedly due to dehydration.

Because some of the inorganic phosphorus values were low, the phosphates were analysed more completely in the latter part of the study. Unfortunately, four of the five cows reported in this study (table 4) represented complicated cases. Three of the plasma acid-soluble inorganic phosphorus values were somewhat low and three of the values for serum phosphatase activity were distinctly low.

DISCUSSION

In general, the values for the various blood lipids, minerals and ascorbic acid of ketotic cows reported in this paper do not deviate markedly from normal values in the early stage of lactation. However, most of the blood lipid values were lower than was to be expected, with the exception of neutral fat and free fatty acids, which increased with the duration of the ketotic condition. Ordinarily, the blood plasma phospholipids and cholesterol esters decrease at parturition and then gradually increase during the second and third week postpartum. Cows with ketosis do not appear to exhibit this normal increase. In the later stages of ketosis, these values are even lower than in the early stages.

The blood mineral values of the ketotic cows were more nearly normal than the blood lipids. The fact that plasma sodium and potassium remained normal indicates that if the adrenal cortex is involved in ketosis, the factor regulating plasma sodium and potassium is not affected. Since the blood serum phosphatase activity was fairly low, it is possible that some abnormalities may exist in metabolic processes where phosphorus is involved. Several normal values for inorganic phosphorus in blood plasma indicate that a phosphorus deficiency is not associated with ketosis.

Since cows with ketosis usually exhibit inappetance, some of the alterations observed in the blood picture may be associated with fasting rather than with ketosis as such. Since little information is available on the effect of fasting in the early postpartal period upon the blood substances studied, such a study appears to be necessary before any further conclusions can be drawn.

SUMMARY

In a study with 18 cows diagnosed as having ketosis, an analysis was made of various blood and blood plasma substances. Plasma phospholipids and cholesterol ester fractions were somewhat low, particularly in the later stages of ketosis. Free cholesterol in the plasma was relatively normal. The amount of free ether-petroleum ether soluble non-volatile acids in plasma determined by acidometric titration was relatively high in the later stages of ketosis. The neutral fat fraction was relatively low in the early stages of ketosis and normal or high in the later stages.

Marked variations were observed in the plasma ascorbic acid values. The serum phosphatase activity was relatively low. The plasma acid-soluble phosphorus values, both inorganic and organic, were sometimes low but were normal

TABLE 3

Hematocrit values, ascorbic acid and minerals in the blood of cows with ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Red cell volume	Plasma ascorbic acid	Plasma Na	Plasma K	Blood Cl	Plasma inorg. P
		(*mg. %*)	(*mg. %*)	(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
				A. Apparently uncomplicated ketosis					
4/20/46	Downey	21.3	32.2	.....	.....	345	19.4	.....	.....
4/16/47	Pelagie	21.6	36.2	.....	.....	307	17.3	.....	.....
3/24/48	Hall	56.2	10.0	36.7	0.062	.....	22.1	307	3.0
3/25/48	Hall	37.5	10.9	35.7	0.063	248	16.8	294	3.3
3/24/48	Downs I	41.9	13.4	36.8	0.175	261	19.8	307	5.5
3/24/48	Downs II	35.1	.....	31.4	0.062	281	22.0	314	3.0
3/25/48	Flegel	39.4	20.5	31.7	0.573	248	20.5	269	6.3
	[a]Av.			34.0	0.218	282	19.8	298	4.2
				B. Complicated ketosis					
3/ 3/48	Hoffman	18.9	40.2	.....	0.549	.....	.....	307	4.1
3/ 5/48	Hoffman	28.9	30.9	.....	0.940	366	.....	310	3.4
3/ 9/48	Thom	56.2	8.3	28.2	0.130	318	18.4	319	.....
3/30/48	Cunningham	24.4	25.1	41.6	0.113	292	15.6	294	4.6
4/ 6/48	Burdette	39.4	5.0	30.0	0.388	252	13.2	248	8.1
4/ 7/48	Burdette	41.2	8.1	34.6	0.275	292	10.1	168	6.4
2/ 7/49	Mullinix	33.7	38.9	34.5	0.338	.....	.....	285	2.7
3/16/49	Thomas	19.3	56.8	.....	0.975	.....	.....	300.5	3.5
3/30/49	Enterprise	22.1	46.2	.....	0.360	.....	.....	275	2.7
	[a]Av.			34.2	0.427	312	15.2	284	4.1

[a] The value for each cow used for calculating the group averages was the average of all individual values.

TABLE 4

Blood phosphates and phosphatase values of cows with ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Blood acid-soluble P			Plasma acid-soluble P			Phosphatase activity	Remarks
				Inorg.	Org.	Total	Inorg.	Org.	Total		
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*units/ 100 ml.*)	
8/16/48	Beltsville 328	22.3	45.0	.....	.....	.....	6.86	0.82	7.68	.....	Apparently uncomplicated ketosis
2/ 7/49	Mullinix	33.7	38.9	2.50	4.30	6.80	2.71	0.83	3.54	1.57	Complicated ketosis
3/16/49	Thomas	19.3	56.8	3.68	2.45	6.12	3.54	1.31	4.85	1.05	Complicated ketosis
3/30/49	Enterprise	22.1	46.2	2.55	3.61	6.16	2.66	1.12	3.78	1.55	
4/28/49	Sherman	17.8	12.8	1.85	5.34	7.19	2.40	1.89	4.29	1.99	Complicated ketosis
	Av.	.....	.....	2.65	3.93	6.57	3.63	1.19	4.83	1.54	

in most cases. Plasma sodium and potassium were normal and the blood chloride values also were in the normal range. Additional data on the effect of fasting and other factors secondary to ketosis are needed before the data can be properly evaluated.

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