CCLV. FASTING AND REALIMENTATION IN THE RUMINANT.

I. THE EFFECT OF FOOD AND FASTING ON CERTAIN BLOOD CONSTITUENTS.

II. CALCIUM AND PHOSPHORUS METABOLISM DURING FASTING, AND DURING REALIMENTATION FOLLOWED BY FASTING.

BY FRANCIS XAVIER AYLWARD¹
AND JANET HENDERSON BLACKWOOD.

From the Hannah Dairy Research Institute, Kirkhill, Ayr.

(Received 8 February 1936.)

I. THE EFFECT OF FOOD AND FASTING ON CERTAIN BLOOD CONSTITUENTS.

IN the course of a study of the precursors of milk fat it became necessary to have detailed information regarding not only the nature and distribution of blood lipins in the cow but also their variation under experimental conditions. The existing information concerning blood lipoids, although largely derived from micro-determinations and therefore to be accepted with reserve, permits certain general conclusions so far as carnivora and non-ruminant herbivora are concerned. It is recognized [Terroine, 1914, 1, 2; 1919] that wide variations in blood lipoids exist between different species and between individual members of the same species although for a particular animal a certain degree of constancy is characteristic.

Of the factors which influence the various constituents of blood, one of the most important is diet; the present work deals with this factor in so far as it affects ruminants.

Feeding with high fat diets has been found to produce an increase in total lipoids in dogs and rabbits [Bloor, 1932]. A similar lipaemia has been observed after a single fatty meal in dogs [Reicher, 1911; Terroine, 1914, 1, 2; 1919; Bloor, 1915, 1916, 1921; Bang 1918 and others], but not in the rabbit [Sakai, 1914; Bloor, 1921; Iwatsuru, 1925], and not invariably in man [Mann & Gildea, 1932].

The results of Maynard & McCay [1929] and of Porcher & Maynard [1930] indicate that alimentary lipaemia is inconspicuous or non-existent in cows, probably owing to the slowness of digestion and absorption in these and other ruminants. It is evident that fasting in such animals must be prolonged in order both to make clear its effects on blood lipoids and to reduce the latter to their basal level.

In addition to a study of the blood lipoids, it is important that the normal values for certain other blood constituents, such as iron, sugar, amino-N and P should be established and that their variations in response to stimuli should be

¹ Agricultural Research Scholar.

determined. It is true that the role of P has been widely investigated, but as yet little has been recorded as to the distribution in blood of the various forms of this element under varying physiological conditions.

With these considerations in view the investigations recorded in the present communication were undertaken.

EXPERIMENTAL.

Methods.

Throughout this work a portion of each sample of blood was used to obtain an alcohol-ether extract in the usual way (1 part blood in 20 parts of 3 : 1 alcohol-ether mixture). Lipin-P was estimated colorimetrically by King's [1932] modification of the Fiske and Subbarow method, cholesterol by digitonin, and total fatty acids by a slightly modified form of the Stoddart and Drury technique. Blood sugar was determined by the improved form of the Hagedorn and Jensen method described by Fujita & Iwatake [1931], amino-N by the colorimetric procedure of Folin [1922], chloride by the Whitehorn [1921] thiocyanate method, and iron by the technique of Blackwood & Stirling [1932]. Calcium was titrated as oxalate using the precipitation technique of McCrudden [1909-10; 1911-12] and the washing methods of Clark & Collip [1925].

Exp. 1. A cow which had been receiving a normal diet was starved for 24 hours and then given a test meal of linseed oil 1, oats 5, straw 7 and flaked maize 1 lb. The fat and P intakes were estimated to be 340 and 8.92 g. respectively, 340 g. fat being equivalent to 0.85 g./kg. body weight. Twelve blood samples were taken alternately from each mammary vein, this procedure being adopted to minimize the inflammation caused by taking a large number of samples over a short period from the same vein. The results recorded in Table I appear to justify this alternate sampling.

Table I. *Two-hourly sampling after a meal. Distribution of various constituents (mg./100 ml. blood).*

Hours before or after meal	Lipin-P	Total fatty acids	Free sterol	Sterol as ester	Iron	Sugar	Amino- acid N	In- organic P	Organic acid- soluble P
Before 3	9.20	225	76.1	64.3	40.3	50.3	8.34	5.55	1.91
1	9.30	227	77.8	55.4	38.9	44.5	7.98	5.60	2.04
After 1	9.49	—	78.7	61.7	39.3	43.6	7.98	5.06	2.38
3	—	—	77.8	—	40.3	37.8	9.37	5.25	2.44
5	9.19	231	78.1	53.1	38.1	44.5	7.24	5.44	—
7	9.60	225	81.6	51.0	42.8	44.2	7.92	4.94	2.88
9	9.71	225	82.6	60.0	40.3	55.9	9.17	5.26	2.53
11	9.42	225	81.3	49.9	41.0	53.3	8.55	4.92	2.60
13	9.67	225	78.4	55.6	39.7	54.4	8.41	5.27	2.54
15	9.51	216	79.7	50.7	40.1	53.3	9.31	4.83	2.58
17	9.56	216	80.0	48.8	38.1	57.0	7.33	4.86	2.76
19	9.40	212	78.4	46.0	37.9	52.1	8.85	4.56	2.73
Mean values	9.46	223	79.2	54.4	39.7	49.2	8.37	5.13	2.49
Standard deviations	0.18	6.7	2.14	3.40	2.61	6.3	1.13	0.28	0.28

The interval between the previous meal and the test meal was 24 hours.

In case a basal level, differing from the normal absorption level, if such exists, might not have been attained in the 24-hour period of fasting previous to the test meal, *Exp. 2* was devised.

Exp. 2. A cow which had previously been on a normal diet was given its final ration at 10 p.m. and was then deprived of food for 5½ days, being allowed

water *ad lib*. Rumination ceased on the 3rd day and about the same time dilatation of the pupils was noticed, but no other ill effects. Care was necessary in realimentation and so immediately after the last fasting sample had been taken, 6 lb. hay were given to induce rumination and promote normal digestion. 4 hours later the following meal was given: linseed oil 2, oats 3, straw 7, flaked maize 1 lb. The fat and P intakes were 529 and 13.0 g. respectively, 529 g. fat being equivalent to 1.32 g./kg. body weight. For the next 3 days 12 lb. hay were given daily, and no difficulty was experienced in realimentation. The times of sampling and the results will be clear from Table II.

Table II. *Blood constituents during 5½ days' fast and after a meal (mg./100 ml. blood).*

Time after last meal (days)	Lipin-P	Total fatty acids	Free sterol	Sterol as ester	Iron	Sugar	Amino-acid N	In-organic P	Organic acid-soluble P
½	8.68	159	79.8	45.8	34.5	46.5	6.65	3.67	2.45
1½	8.36	161	78.8	48.4	38.3	36.9	4.53	3.76	2.20
2½	7.56	160	75.8	43.4	39.7	42.8	6.62	4.33	2.73
3½	7.89	160	76.2	51.0	35.2	35.9	4.97	5.46	2.64
4½	7.57	165	76.4	49.6	36.2	49.3	5.36	5.87	2.91
5½	7.57	149	75.4	45.4	40.0	51.9	6.09	6.78	2.86
Time after experimental meal (hours)*:									
8	7.60	156	75.6	51.2	37.7	51.7	7.53	7.39	3.14
20	7.53	135	74.6	43.0	38.5	44.4	9.46	6.93	3.64
32	7.92	135	73.6	51.2	35.5	43.8	9.94	7.28	3.51
44	7.52	137	69.2	49.6	32.6	43.3	8.82	7.46	2.38
68	7.40	132	70.6	58.2	33.9	42.2	9.20	5.95	2.66
8 days	7.54	130	67.9	52.9	31.9	44.7	6.85	5.72	2.80

* The test meal was given 4 hours after the previous sample had been taken. The next sample was obtained 8 hours after the administration of the meal.

Table III. *Constituents of blood during a 7½-day fast recorded (in brief), and during a 10½-day fast (recorded in detail) (mg./100 ml. blood).*

Time before or after meal	Lipin-P	Total fatty acids	Free sterol	Sterol as ester	Iron	Sugar	Amino-acid N	Ca	Cl	In-organic P	Organic acid-soluble P
Exp. III:											
12 hours before	10.34	204	89.1	51.4	48.5	47.0	7.66	5.73	—	4.17	2.91
12 hours after	10.21	191	92.6	54.6	47.0	38.6	7.08	6.01	—	3.63	2.65
2½ days after	9.74	192	91.1	52.1	47.0	45.1	8.36	6.15	—	3.60	3.12
4½ days after	9.86	208	96.6	46.0	49.3	49.7	8.44	6.59	—	4.19	3.28
7½ days after	9.54	179	94.4	36.1	49.3	68.6	6.54	5.85	—	4.01	3.56
Meal given immediately											
24 hours later	9.81	211	95.8	37.9	56.5	68.6	5.99	5.81	—	4.23	3.91
48 hours later	10.20	—	98.1	34.7	51.9	70.5	—	5.93	—	3.82	3.61
Exp. IV:											
12 hours before	9.44	198	92.4	57.9	41.7	52.2	6.07	6.70	268	3.86	3.37
12 hours after	10.04	203	95.9	39.0	37.1	71.9	7.05	7.33	267	3.94	2.58
1½ days after	10.37	214	96.3	37.4	43.5	65.5	7.12	7.21	261	4.92	2.35
2½ days after	10.27	—	98.5	54.7	37.1	65.1	5.10	6.43	266	4.72	3.27
3½ days after	10.06	224	95.7	58.0	43.9	44.9	—	6.75	279	4.95	3.19
4½ days after	9.56	186	91.1	59.2	—	50.5	5.81	6.63	276	5.08	3.12
5½ days after	9.54	201	87.6	48.1	41.4	52.4	6.23	6.40	280	4.86	2.99
6½ days after	9.39	204	87.5	46.3	—	51.9	6.12	6.75	276	4.61	2.55
7½ days after	8.70	207	85.0	53.9	43.4	47.3	6.88	6.32	265	4.53	2.66
8½ days after	8.25	207	91.4	37.4	—	49.9	6.84	6.63	271	4.44	2.63
9½ days after	8.89	213	89.3	46.2	—	49.1	5.10	5.89	267	3.95	2.96
10½ days after	8.36	176	89.1	40.6	43.4	46.5	6.88	6.90	267	3.76	2.86
Meal given immediately											
24 hours later	8.33	178	84.7	39.6	38.3	38.0	8.26	6.55	268	3.74	3.03

Previous to the fast the animals were on a low protein diet for a week.

Exps. 3 and 4. Two non-lactating cows were subjected to periods of $7\frac{1}{2}$ - and $10\frac{1}{2}$ -day fasts respectively, and in view of the paucity of starvation experiments on ruminants, metabolism trials were run and will be described later. Blood samples were taken from alternate subcutaneous abdominal veins at daily intervals. The animals finished the experimental periods in good condition. The results are set out in Table III.

DISCUSSION.

In Table I it is clear that the ranges of variation of all the constituents studied are extremely small, the contrast between the mechanism of digestion and absorption in the ruminant and that in one-stomached animals being most obvious. The standard deviations are recorded and these may be used in subsequent experiments to test the significance of any apparent changes over a similar period.

In view of the results of Channon & Collinson [1929, 1, 2] indicating that the glyceride fraction of blood fat may be very small or even absent, the distribution of the lipoids was calculated, using the factors generally adopted. On this basis the mean values recorded for the fatty acids in Table I suggested the following distribution: as phosphatides 172.6, as steryl esters 39.6, and as glycerides 10.5 mg. fatty acids per 100 ml. blood. That the figure for the glycerides is exceedingly low or zero can readily be confirmed in the same way from the other three experiments. These findings are therefore in agreement with those of Channon & Collinson, but it must be realized that the factors used are those adopted mainly for liver phosphatide and may not be strictly applicable to blood, which contains a higher proportion of sphingomyelin. In fact Lintzel [1934] has suggested the factor 16.5 to 16.8 for converting blood lipin P to phosphatide instead of 18.5 as used for liver. Lintzel also finds an appreciable difference between the total alcohol-ether-soluble P and the acetone-precipitated P. As the identity and magnitude of the glyceride fraction of blood deserve further study in view of the possible importance of these compounds in the secretion of milk fat, the whole question is receiving further attention.

During the much longer fasting period of Exp. 2, there appeared to be a significant decrease in lipin P, realimentation restoring the initial level. There was no significant change, however, in the total fatty acid and sterol fractions which did not show any tendency to reach a basal level markedly dissimilar from the normal.

In Exps. 3 and 4, both cows showed a decrease in lipin P about the 3rd day of starvation, the decrease becoming more marked in the later stages particularly with the second animal. Similar results have been recorded for the dog [Ling, 1931], pigeon [Müller, 1929], rat [Sure *et al.*, 1933], and contrary results for the rabbit [Iwatsuru, 1925].

There also appears to be some tendency in these two experiments for the sterol to diminish, but the series is not without anomalous values.

With regard to iron, sugar and amino-N, the deviations from the initial values were somewhat larger than the standard deviations of Exp. 1, but no general trend was shown except perhaps in the long starvation, Exp. 4, where the sugar level may have reached a stable value. The variations in Cl content found in Exp. 4 were very small.

Phosphorus distribution. The variations in inorganic and organic acid-soluble P are shown in Tables II and III. From Table II it will be seen that the inorganic P increases regularly during the starvation period, the maximum value being reached 44 hours after realimentation. The changes in organic acid-soluble

P are approximately parallel. Blatherwick [1920] in a single 7-day fasting experiment on a cow observed slight increases in the total and inorganic P contents of the plasma. These he ascribed mainly to the mobilization of mineral reserves to assist in the maintenance of tissue neutrality. No evidence of acidosis was found. Gato [1922] in experiments on fasting rabbits also records changes in blood P. Increases in the inorganic P of the blood during starvation have also been observed by Cavins [1924] and by Wilder [1929] working on rachitic rats.

The organic acid-soluble P increases fairly regularly throughout fasting to a maximum observed about 20 hours after the first post-fasting meal, but the changes are not very great when referred to the standard deviations of Exp. 1. In the present experiments the range found is 3.7–7.5 mg. per 100 ml. blood for inorganic P, 2.2–3.6 for organic acid-soluble P and 6.0–10.8 mg. for total acid-soluble P.

It is of interest to discuss the reason for these increases in inorganic and organic acid-soluble P and also the source of the excess P. During starvation it is well known that extensive tissue breakdown occurs for, in addition to the loss of protein due to the normal wear-and-tear of the organism, energy requirements have to be met, in part at least, from body protein. Oxidative degradation of the phosphoproteins will throw simpler forms of P into the blood-stream and if this is not excreted as rapidly as it is produced, it will be reflected in high levels of the P components of the blood. In addition to the protein of the tissues, the creatinephosphate which is known to be present in muscular tissue is a possible source of organic acid-soluble P.

A second and equally important source of P may be found in the bone phosphates. This is a factor which cannot be ignored when it is remembered that 90 % of the total P of the body is in the skeletal tissue. Utilization of this store has frequently been referred to, particularly in lactating cows. The dephosphorylation of bone would, of course, be accompanied by a mobilization of Ca. Three methods of approaching this problem present themselves. (1) If protein degradation is associated with the rise in blood P, inorganic sulphate in the blood might be expected to show a similar and related increase. Data regarding the inorganic sulphate content of the blood might then be of value. (2) If demineralization of bone occurs during fasting simultaneous increases of blood P and Ca might be observed if no compensatory mechanism is involved. (3) Information about the normal metabolism of the ruminant may be most readily acquired by the examination of the excreta. Extensive catabolism either of bone or of soft tissue ought to be revealed by determination of the output of N, P and Ca.

The second method was followed in experiments subsequent to Exp. 2 and the results are reported in Table III. Concurrently the third method was adopted and the results are reported in Part II.

Ca and P distribution in Exps. 3 and 4. A scrutiny of the results for blood Ca (Table III) shows that there are no consistent changes throughout the starvation period, although the range of variation is rather wide. Thus if demineralization of bone does take place during fasting there must be an efficient mechanism securing the maintenance of the normal blood Ca level.

As before, increases in inorganic P and total acid-soluble P may be observed during fasting, the maximum values being attained on the 6th day in Exp. 3, and on the 5th day in Exp. 4. The increases are not so marked as those of Exp. 2, but evidence that the maximum values are genuine was obtained from the metabolic studies which will be reported later. The smaller increases may be due to the fact that the three animals were in different physiological states. Cow No. 2 was in the 6th month of gestation whereas the others were non-pregnant. The

pregnant fasting animal has not only to obtain energy for its own maintenance but has also to provide energy and nutriment for the growth of the foetus. The foetus will require not only fat, protein and carbohydrate for its growth, but also minerals, and the requirements of the latter (especially of Ca and P) will be very high from the 5th month onwards [Turner, 1931]. From this point of view, and in the absence of new sources of P, a tendency for the blood P of the maternal circulation to decrease rather than increase might be expected. Some other factor such as extensive catabolism of P-containing material would therefore seem to be involved.

II. CALCIUM AND PHOSPHORUS METABOLISM DURING FASTING, AND DURING REALIMENTATION FOLLOWED BY FASTING.

The object of this part of the work was to study the Ca and P excretions during fasting in relation to the blood P in the hope that such an investigation might reveal the cause of the increased mineral metabolism during starvation. The urinary analysis of Benedict's fasting experiment [Benedict & Ritzmann, 1927; Carpenter, 1927], although fairly complete with respect to N and S, contained only a few P analyses. Determinations were made of fixed bases but not of Ca, and the analyses of faeces during fasting were confined to dry matter and to total N. In the present paper the rebuilding of both the protein and the skeletal tissue has been followed, while the relationship between blood inorganic P levels and urinary P excretion has also been investigated. The work is divided into three sections.

SECTION A.

During Exps. 3 and 4 already described, urinary and faecal P and faecal Ca were estimated. The results are set out in Table IV.

Table IV. *Ca and P excretion (g./day).*

Period	Days of fast	Exp. III				Exp. IV			
		Urine		Faeces		Urine		Faeces	
		P	N/P	Ca	P	P	N/P	Ca	P
Prefasting	—	—	—	—	—	0.07	313	14.69	4.53
	—	—	—	—	—	0.07	265	13.99	5.71
	—	—	—	—	—	0.06	248	14.08	5.72
	—	0.04	359	9.72	5.45	0.09	233	13.44	6.72
	—	0.10	108	12.82	4.53	0.08	244	7.37	6.43
	—	0.10	155	13.08	4.81	0.12	222	12.13	7.22
	—	0.07	185	11.17	5.13	0.13	200	11.70	5.85
	—	0.09	133	19.46	7.34	0.06	233	9.83	6.00
	—	0.09	103	11.75	4.34	0.09	286	8.01	5.50
Early fasting	1½	0.14	106	13.16	5.88	0.11	179	14.82	6.84
	2½	0.18	110	4.39	2.25	0.09	378	7.85	3.59
	3½	0.14	142	5.04	1.98	0.07	609	5.35	4.17
	4½	0.13	220	3.08	1.10	0.13	322	5.62	2.80
Effective fasting	5½	0.48	57.4	2.68	0.88	0.23	152	2.65	2.76
	6½	2.81	17.6	2.66	0.85	0.48	67.1	1.29	1.09
	7½	4.46	12.1	3.61	0.91	2.46	16.3	3.44	4.00
	8½	—	—	—	—	4.27	10.9	13.48	4.60
	9½	—	—	—	—	3.99	11.0	4.64	3.71
	10½	—	—	—	—	3.72	11.6	3.12	4.01

The Ca and P intakes in the prefasting period were 14.0 and 6.90 g./day respectively in both experiments.

It is of interest to observe that the urines of the cows in the four fasts remained alkaline. This was contrary to the results of Carpenter [1927] where the urine of steers though initially alkaline became acid after a few days.

Urinary P. The P percentage and total daily excretion of P in the urine are remarkably low in the preliminary period (low protein diet) and during the first 4 days of starvation. The P concentration in the urine never exceeds 5 mg./100 ml. and the total daily output is always less than 200 mg. Thus from the point of view of P excretion fasting is commenced from a definite equilibrium marked by a definite basal urinary output. These low figures are similar to those obtained by Meigs and his co-workers [1919; 1926], and much lower than those found for the human subject. The urinary P output of man, although largely dependent on the diet, is usually about 2 g. per day. In the calf before rumination begins, the output of urinary P is higher than that of cows. This difference may possibly be associated with the type of digestion and absorption characteristic of ruminants and in this connection it may be mentioned that ruminants also differ markedly from non-ruminants in the distribution of the P compounds in the blood, the organic acid-soluble P fraction of the former being particularly low [Blackwood, 1934]. The low level of urinary P excretion is maintained for the first 4 days of fasting. This is in accord with the general conceptions of ruminant digestion. On the 5th and subsequent days, however, a marked change occurs, characterized by progressive increases in percentage and total output of P. The levels attained are similar to those obtained in carnivorous animals including man where fasting is accompanied by a continuous excretion of P. Benedict [1907; 1915] finds that the P output continues in man even throughout long fasts and at the 31st day in one experiment 1.3 g. P_2O_5 were excreted. Occasionally also, a rise in urinary P was observed during the first few days of fasting. Similar results are recorded by Cathcart [1907]. The source of this continued excretion of P during fasting, whether from bone or muscle tissue, can only be determined by correlation with the N and Ca outputs. The time of the rise in urinary P excretion is coincident with the maximum value of inorganic and total acid-soluble P in the blood. This suggests a close relation between the two phenomena. A proportionality between plasma phosphate and urinary phosphate excretion has been postulated by Addis *et al.* [1925] under certain restricted conditions. The blood P therefore appears to increase to a limiting value, after which increased urinary excretion commences.

*Correlation of N and P outputs in urine.*¹ If muscle protein alone is the source of the urinary P then one would expect the ratio of urinary N to P found, after the exogenous output of these elements has ceased, to approximate to that of muscle itself, namely 17.4 [Lusk, 1931]. The ratio found in the experiments is given in Table IV. The original value of the ratio is seen to be very high and these high values are maintained during the first 4 days of starvation. An extraordinarily rapid fall then takes place. In Exp. 3, owing to the shortness of effective fasting, no equilibrium is attained. The ratio, however, on the last day of the fast is 12.1. In Exp. 4, equilibrium is established at a value approximating to 11.2 from the 8th day.

It is clear then that catabolism of some P-containing material in addition to protein has taken place. Similar low ratios have been found by Cathcart [1907] and other authors and have been generally ascribed to demineralization of bone. A further explanation might rest in the fact that the urine may not be the only excretory channel for P during starvation. Before discussing this question, how-

¹ The N metabolism of these fasts has been included in a paper by Hutchinson & Morris [1936] from which we quote the figures for urinary N.

ever, the figures for faecal Ca and P must be considered. It may be noted here that no urinary Ca determinations are recorded since previous experience and subsequent experiment show that such excretion is normally negligible and shows no rise during starvation.

Faecal P and Ca. During the preliminary period of Exps. 3 and 4 the daily faecal excretion of P represents a fairly uniform basal level, while the percentage composition of the dry faeces shows remarkably small variations. In Exp. 3 this percentage composition (*ca.* 200 mg.) is maintained throughout the entire fasting period. The daily output, however, decreases rapidly to an equilibrium level of about 0.9 g. In Exp. 4 the percentage composition of the dry faeces rises rapidly during starvation. In the early days of starvation a fall in faecal output of dry matter is accompanied by a decrease in the faecal P output. Thereafter, however, the P output rises sharply and almost reaches the prefasting level. The salient feature of each experiment is, then, the continued faecal excretion of P during starvation. In the later stages of fasting this P can only arise from endogenous sources.

The Ca percentage in the dry faeces in each experiment shows no marked difference between the prefasting and starvation periods. The total output, however, although falling rapidly from the prefasting level never becomes insignificant. Thus in the last 3 days of Exp. 3, 8.95 g. of Ca are eliminated and in the last 6 days of Exp. 4, 28.62 g. This, too, can only be of endogenous origin.

This continued excretion of Ca and P in starvation faeces has also been observed by Wellmann [1908] in rabbits.

The main source of the endogenous Ca excretion must be the bone. Such demineralization must result in a simultaneous liberation of P so that part, at least, of the P excreted either in the urine or faeces must arise from this source. The presence of P derived from bone in the urine, in addition to that from protein, is an adequate explanation of the low N/P values observed in the urine.

It has been accepted for some time that Ca can be eliminated from the bloodstream by re-absorption in the lower intestine. Excretion of Ca by the kidney can only take place in the form of soluble salts [Holt *et al.*, 1925]. This excretion will be governed partly by the solubility products of the salts themselves and partly by the reaction of the urine. The urine of the cow is normally highly alkaline and this fact explains why simultaneous large excretions of Ca and P do not take place via the kidney. Such simultaneous eliminations are necessary during fasting, and in view of the maintenance of the alkalinity of the urine in these experiments, one can only assume that re-excretion of both Ca and P into the bowel is possible. This is in agreement with the views of Shelling [1932].

Phosphorus balance sheet. (a) *Protein.* Assuming that the urinary N in the later stages of the fast is entirely endogenous and derived from body protein, the P from the same source may be calculated. $P \text{ derived from protein} = \frac{\text{urinary N}}{17.4}$ where $17.4 = \frac{N}{P}$ in muscle. (b) *Bone.* It is generally accepted that 85% of the Ca of bone is present as calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), the remainder being mainly carbonate [Shear & Kramer, 1928]. The amount of Ca found in the soft tissues of the body is usually very small. Assuming as before that the Ca in the later stages of the fast is derived from bone, then $\text{Ca derived from bone phosphate} = \text{total Ca excreted} \times 0.85$, and so $P \text{ derived from bone} = \frac{\text{total Ca} \times 0.85}{1.93}$ where $1.93 = \frac{\text{Ca}}{P}$ as in $\text{Ca}_3(\text{PO}_4)_2$. Making these calculations on the data of the fasts from the fourth day onwards, part of Table V was drawn up.

Table V. *P balance sheets for effective fasting period (g./day).*

Exp.	Days of fast	Urinary N	P from protein	P from bone	Total P excretion by calculation	Total P excretion actually found
III	5½	27.32	1.86	1.18	3.04	1.36
	6½	49.45	3.36	1.17	4.53	3.66
	7½	54.12	3.68	1.59	5.27	5.37
				Total	12.84	10.39
IV	5½	34.98	2.38	1.17	3.55	2.99
	6½	32.21	2.19	0.57	2.76	1.57
	7½	40.76	2.77	1.51	4.28	6.46
	8½	46.71	3.18	5.94	9.12	8.87
	9½	43.96	2.99	2.04	5.03	7.72
	10½	42.76	2.91	1.37	4.28	7.73
				Total	29.02	35.34
V	5	35.28	2.03	0.76	2.79	1.54
	6	67.94	3.90	0.52	4.42	3.96
	7	23.59	1.36	0.16	1.52	0.99
	8	32.34	1.86	0.47	2.33	1.65
	9	25.58	1.47	0.55	2.02	3.06
				Total	13.08	11.20
VI	5	52.02	2.99	2.55	5.54	2.97
	6	48.18	2.77	2.07	4.84	2.27
	7	49.49	2.84	1.31	4.15	2.58
	8	None	None	2.07	2.07	0.45
	9	65.50	3.76	1.11	4.87	4.97
	10	100.30	5.76	1.68	7.44	7.51
	11	None	None	5.11	5.11	0.33
	12	117.40	6.75	2.69	9.44	9.66
				Total	43.46	30.74

The total P excretion calculated is in fairly close agreement with that found and this is a test of the validity of the factors used and of the assumptions made. We regard this therefore as strong evidence that during prolonged fasting continuous mobilization of bone and protein P takes place.

SECTION B.

After the completion of the fasts in Exps. 3 and 4, the cows were placed on a high protein diet until N equilibrium was established. A low protein ration was then given followed by the original high protein diet, the change over being again determined by the attainment of N equilibrium values. The P intakes on the high and low protein diets were 15.6 and 8.9 g./day respectively. Blood samples were taken from alternate mammary veins at intervals and the P distribution determined. The results are given in Tables VI and VII.

Blood levels. Each cow finished the fasts of Exps. 3 and 4 with comparatively low blood levels of inorganic P, namely 3.82 mg. and 3.74 mg. The immediate response to a high protein diet was a large increase in this level, the mean values during this period being 5.23 and 5.35 mg. The organic acid-soluble P showed no significant change. In the low protein period levels prevail with means of 4.53 mg. and 4.35 mg. In the subsequent high protein period little change in these values is observed. The experiments of Robinson & Huffmann [1926] and Huffmann *et al.* [1933] have shown that the amount of P in the diet has a marked influence on the P content of the blood. Two possibilities present themselves. (1) The differences in the daily P intakes 15.64, 8.90 and 15.64 g. may not be sufficiently great to bring about the changes noted by these authors. Our figures were, of

Table VI, Exp. III. *P* excretion, and also *P* distribution in the blood, on high and low protein diets.

Diet and P intake	Days	P excretion (g./day)			P in the blood (mg./100 ml.)		
		Faeces	Urine	Retention	Inorganic	Organic acid- soluble	Lipin
High protein 15.64 g. P per day	1	26.49	2.33	-13.18	—	—	—
	2	16.89	2.05	-3.30	—	—	—
	3	13.86	1.93	-0.15	—	—	—
	4	8.02	1.13	6.49	6.58	1.76	9.36
	6	11.19	1.32	3.13	5.48	2.46	9.60
	8	12.38	0.61	2.65	4.60	2.61	9.28
	9	11.24	0.39	4.01	—	—	—
	10	9.41	0.34	-0.85	4.28	1.81	9.76
	12	14.42	0.17	-5.69	4.33	2.31	9.76
Low protein 8.90 g. P per day	14	6.30	0.28	2.32	4.85	2.42	9.92
	16	4.73	0.21	3.96	4.35	2.59	10.16
	18	7.86	0.17	0.87	4.49	3.12	10.00
	20	5.07	0.19	3.64	4.93	2.75	10.01
	22	8.61	0.11	0.18	4.45	2.78	10.00
	24	7.24	0.14	1.52	4.42	3.28	9.74
	26	9.44	0.10	-0.64	4.52	2.43	9.77
	28	5.18	—	3.72	4.46	2.23	9.93
	29	5.47	0.18	9.99	4.55	3.00	10.39
High protein 15.64 g. P per day	31	8.21	0.19	7.24	4.85	1.56	9.00
	33	7.22	0.12	8.30	—	—	—
	35	8.60	0.07	6.97	4.28	2.58	—
	36	9.68	0.08	5.88	4.45	—	8.83
	Mean values:	High protein			5.56	2.28	9.41
		Low protein			4.51	2.57	9.91
		High protein			4.53	2.38	9.41

Table VII, Exp. IV. *P* excretion, and also *P* distribution in the blood, on high and low protein diets.

Diet and P intake	Days	P excretion (g./day)			P in the blood (mg./100 ml.)		
		Faeces	Urine	Retention	Inorganic	Organic acid- soluble	Lipin
High protein 15.64 g. P per day	1	0.60	0.12	14.92	5.85	2.06	9.69
	3	8.66	0.12	6.86	5.21	1.80	9.60
	5	4.43	0.11	11.10	5.30	1.92	9.54
	7	7.06	6.23*	2.35	5.90	2.09	9.65
	9	8.69	0.11	6.84	5.00	2.39	9.71
	11	11.46	0.10	4.08	5.80	2.94	9.47
	13	5.61	0.13	9.90	5.26	3.00	9.64
	15	8.46	0.09	7.09	5.25	3.13	9.51
	17	11.64	0.08	3.92	5.32	3.07	10.11
	19	8.65	0.09	6.90	4.62	3.27	9.72
	20	7.92	0.07	7.65	—	—	—
Low protein 8.90 g. P per day	21	7.39	0.08	1.43	4.80	3.08	10.05
	23	10.13	0.14	-1.37	4.74	2.85	9.82
	25	7.90	0.06	0.37	4.50	2.51	10.48
	27	11.07	0.11	-2.18	4.34	2.66	9.53
	29	9.23	0.10	-0.43	3.90	2.74	—
	31	6.41	0.10	2.39	3.90	2.96	9.36
	33	5.89	0.07	2.94	4.29	2.55	9.72
	34	8.00	0.13	7.51	4.28	2.20	9.80
	36	8.21	0.17	7.26	3.82	2.96	10.88
High protein 15.64 g. P per day	37 + 38	14.15	0.09	1.40	5.15	2.62	9.16
	40	8.48	0.11	7.05	4.45	2.56	10.84
	42 + 43	20.96	0.14	5.09	4.43	3.17	11.60
	44 + 45	24.06	0.18	3.52	—	—	—
	Mean values:	High protein			5.35	2.56	9.66
		Low protein			4.35	2.77	9.83
		High protein			4.41	2.70	10.46

course, regulated by the protein level desired. (2) The previous nutritional state of the animals was such that the claims of replacement of body tissue would be paramount.

Urine. During the high protein period of Exp. 3 urinary P is excreted at a high level. Progressive decreases in percentage composition and total output are noted and at the end of the period a normal excretion level is attained. This may represent a descent from the high levels observed during fasting. A remarkable constancy in these levels persists throughout the subsequent periods. In Exp. 4 a similar low level prevails even throughout the initial high protein period. The value marked with an asterisk in Table VII is inexplicable.

Faeces. The total P output in the faeces is somewhat irregular but usually no very great variations occur. The values in the high protein period of Exp. 3 are above those observed in the subsequent high protein period.

Retention. In Exp. 3 satisfactory retention of P was not established until the second period of high protein diet. In Exp. 4 the first high protein level was sufficient to establish a positive P balance. This, however, could not be maintained on the low protein diet but was re-established on changing to the high protein level.

Probably a number of factors are involved in the restoration of bone tissue, including the amounts of Ca and P absorbed, the ratio of assimilated Ca and P, the excretion of these elements and the physico-chemical conditions prevailing at the site of deposition of bone substance. The delayed or unsatisfactory realimentation observed may be due to failure to satisfy the requirements of all of these factors. In addition, phosphoprotein must also be restored.

SECTION C.

An opportunity was afforded by the continuation of the fasting experiments already described to make a further study of the P metabolism. The two cows were put out to grass following realimentation. They were then fasted for further periods of 10 and 12 days respectively. The results are reported in Table VIII (Exps. 5 and 6).

Blood and urinary P. It is at once apparent that neither cow started the fast with a normal level of blood inorganic P, the initial values being 7.48 mg./100 ml. and 6.50 mg./100 ml. These extremely high values may be associated with the prefasting diet of fresh young grass, which, being rich in minerals, would secure effective realimentation, and it was not to be anticipated that fasting could result in the production of higher levels. It was therefore thought of interest to continue the study of blood and urinary P in these somewhat unusual cases with the object of testing the validity of the views already expressed on the relation of these two P levels.

The high blood P level is maintained for the first 4 days of fasting and is accompanied by a fairly high level of urinary P. This is especially noticeable in the percentage composition of the urine.

Thereafter with Exp. 6 somewhat lower and normal levels of blood P prevail, variations about the mean value of 4.99 being small. The urinary excretion of P, however, rises in precisely the same fashion as in the earlier fasting experiments. On the final day of the fast the P excretion reached the abnormally high value of 9.6 g. while the percentage composition was 141 mg./100 ml. This rise in urinary P again illustrates the degradation of body P. The efficiency of the renal excretion throughout the entire fasting period has thus prevented any temporary accumulation of inorganic P in the blood, so that no rise in level comparable with

Table VIII. *Blood P and excretion data during 10½-day and 12-day fasts.*

Exp.	Days of fast	Blood P (mg./100 ml.)			Day of fast	Ca and P excretion (g./day)			
		In-organic	Organic, acid-soluble	Lipin		Urine		Faeces	
						P	Ca	P	Ca
V	1½	7.48	4.50	9.38	1	0.33	0.14	4.73	21.98
					2	1.89	0.25	5.18	11.00
	3½	7.65	4.04	9.22	3	0.78	0.10	1.58	2.72
					4	1.04	0.07	1.46	1.82
	5½	4.56	3.69	10.46	5	0.22	0.04	1.32	1.69
	6½	3.79	4.87	9.46	6	2.90	0.09	1.07	1.10
	7½	3.77	4.47	9.82	7	0.44	0.18	0.56	0.19
	8½	3.26	5.20	9.97	8	0.32	0.14	1.33	0.92
	9½	2.62	4.53	10.43	9	0.14	0.08	2.92	1.16
	10½*	4.43	4.35	12.60	—	—	—	—	—
VI	1½	6.50	4.09	—	1	2.95	0.31	1.34	18.20
					2	1.26	0.07	0.69	10.50
	3½	7.55	4.25	—	3	1.17	0.10	1.42	15.08
					4	1.68	0.07	0.37	4.44
	5½	5.34	3.86	—	5	2.38	0.25	0.60	5.54
	6½	4.86	3.67	—	6	1.81	0.19	0.46	4.51
	7½	4.70	4.06	—	7	2.24	0.03	0.34	2.95
	8½	5.17	4.33	—	8	None	None	0.45	4.70
	9½	4.12	3.54	—	9	4.58	0.11	0.39	2.41
	10½	5.08	3.88	—	10	7.04	0.13	0.47	3.68
	11½	4.92	3.62	—	11	None	None	0.33	1.16
	12½	5.10	3.97	—	12	9.59	0.16	0.07	0.45

* Jugular post mortem sample.

those of the earlier fasts occurs. It is also noticeable that the fall in blood P level is coincident with the marked rise in the urinary P percentage and this points to the establishment of an equilibrium at the renal tubules. Further consideration of the urinary P of Exp. 5 will be given later.

Ca excretion. It has already been noted that urinary Ca is invariably low. This has been determined throughout the two fasts recorded and shows no significant change. The faecal Ca output declines from its initial high values, a result which may be due to grass feeding, but the output is maintained at a steady level, indicating again a continuous mobilization of bone tissue.

Faecal P. Faecal P output is also maintained at an appreciable level even in the later fasting period, although decreasing from its original figure. If a balance sheet is struck of the mobilization of P reserves in the body on the lines already indicated the part played by protein and bone respectively in the maintenance of bodily activity may be calculated. The data of Table V pertaining to the present experiments have been drawn up on these lines. The agreement between the calculated and observed values of P excretion is good in Exp. 5 but in Exp. 6 a low urinary output, suggesting a suppression of urine and a formation of urinary calculi in the later days of the fast, leaves a fairly large proportion of the expected P excretion unaccounted for. Whilst protein is seen to contribute the larger part of the P excreted, the utilization of the skeletal tissue P is remarkably rapid in onset and its magnitude is not inconsiderable. One interesting feature of these

Exp. no.	P derived from protein g./day (mean)	P derived from bone g./day (mean)
3	2.97	1.31
4	2.85	2.10
5	2.12	0.49
6	3.11	2.32

balance sheets is the agreement in three of the fasts between the relative utilization of protein and bone P and the dissimilarity of the fast of Exp. 5.

Exps. 3 and 5 were carried out on one animal and Exps. 4 and 6 on another. In Exp. 3 there is a suggestion that the mineral reserve was not capable of mobilization to the extent seen in Exps. 4 and 6 and in the second fast with this animal the failure of bone mobilization is most evident. This failure was characterized by marked symptoms of tetany, necessitating the destruction of the animal, and if reference is made to Table VIII the clinical picture is one of very low blood inorganic P accompanied by a diminished urinary P excretion. Presumably investigation would also have shown a low blood Ca.

It appears then that during fasting effective and continuous mobilization of Ca and P from skeletal tissue is necessary, not only to maintain the level of these constituents in the blood but also to aid in the maintenance of tissue neutrality, to preserve a renal equilibrium and possibly to facilitate the conversion and transport of body reserves to meet energy requirements.

GENERAL SUMMARY.

A study of various constituents of blood during fasting and after meals of varying fat content is reported. With the exception of lipin and of acid-soluble P, no constituent showed any consistent change in level. Lipin P was decreased by effective periods of fasting, whilst inorganic and organic acid-soluble P were markedly increased, indicating extensive catabolism of body P. The source of this P is discussed. It is concluded that, apart from the two types of P, the level of the blood constituents investigated is not affected in the ruminant by the processes of digestion and absorption.

Continuous excretion of Ca and P was found during effective fasting of ruminants. The blood P level is correlated with the urinary P output. The source of the continuous excretion of P is endogenous and is shown to be derived from bone and phosphoprotein material. The necessity for this dual mobilization is discussed and an interesting case of failure to maintain effective mobilization is reported.

We are particularly indebted to Mr A. B. Fowler and Mr S. J. Edwards who obtained the blood samples. It is a pleasure to acknowledge the helpful interest of Prof. H. J. Channon, Dr N. C. Wright and Dr J. A. B. Smith.

One of us (F. X. A.) is indebted to the Agricultural Research Council for the grant of a scholarship during the tenure of which this work was carried out.

REFERENCES.

- Addis, Meyers & Bayer (1925). *Amer. J. Physiol.* **72**, 125.
 Bang (1918). *Biochem. Z.* **91**, 104.
 Benedict (1907). *Publ. Carneg. Instn.* No. 77.
 — (1915). *Publ. Carneg. Instn.* No. 203.
 — & Ritzmann (1927). *Publ. Carneg. Instn.* No. 377.
 Blackwood (1934). *Biochem. J.* **28**, 1346.
 — & Stirling (1932). *Biochem. J.* **26**, 353.
 Blatherwick (1920). *J. biol. Chem.* **42**, 517.
 Bloor (1915). *J. biol. Chem.* **23**, 317.
 — (1916). *J. biol. Chem.* **24**, 447.
 — (1921). *J. biol. Chem.* **49**, 201.
 — (1932). *J. biol. Chem.* **95**, 633.
 Carpenter (1927). *Amer. J. Physiol.* **81**, 519.
 Cathcart (1907). *Biochem. Z.* **6**, 109.
 Cavins (1924). *J. biol. Chem.* **59**, 237.
 Channon & Collinson (1929, 1). *Biochem. J.* **23**, 663.
 — — (1929, 2). *Biochem. J.* **23**, 1212.
 Clark & Collip (1925). *J. biol. Chem.* **63**, 461.
 Folin (1922). *J. biol. Chem.* **51**, 377.
 Fujita & Iwatake (1931). *Biochem. Z.* **242**, 43.
 Gato (1922). *Tohoku J. exp. Med.* **3**, 194.
 Holt, La Mer & Chown (1925). *J. biol. Chem.* **64**, 509.
 Huffmann, Robinson, Duncan, Lamb & Mason (1933). *J. Dairy Sci.* **16**, 203.
 Hutchinson & Morris (1936). *Biochem. J.* **30**, 1682, 1695.
 Iwatsuru (1925). *Pflüg. Arch. ges. Physiol.* **208**, 41.
 King (1932). *Biochem. J.* **26**, 292.
 Ling (1931). *Chin. J. Physiol.* **5**, 381; cited by Sinclair (1934), *Physiol. Rev.* **14**, 351.
 Lintzel (1934). *Z. Zücht. B.* **29**, 219.
 Lusk (1931). *The Elements of the Science of Nutrition*, 4th ed., p. 93. (Philadelphia and London: Saunders and Co.)
 Mann & Gildea (1932). *J. biol. Chem.* **99**, 61.
 Maynard & McCay (1929). *J. Nutrit.* **2**, 67.
 McCrudden (1909–10). *J. biol. Chem.* **7**, 83.
 — (1911–12). *J. biol. Chem.* **10**, 187.
 Meigs, Blatherwick & Cary (1919). *J. biol. Chem.* **40**, 469.
 — Turner, Harding, Hartman & Grant (1926). *J. agric. Res.* **32**, 833.
 Müller (1929). *J. biol. Chem.* **84**, 345.
 Porcher & Maynard (1930). *Lait*, **10**, 765.
 Reicher (1911). *Verh. Kongr. inn. Med.* **28**, 327.
 Robinson & Huffmann (1926). *J. biol. Chem.* **67**, 245.
 Sakai (1914). *Biochem. Z.* **62**, 387.
 Shear & Kramer (1928). *J. biol. Chem.* **79**, 105.
 Shelling (1932). *J. biol. Chem.* **96**, 229.
 Sure, Kik & Church (1933). *J. biol. Chem.* **103**, 417.
 Terroine (1914, 1). *J. Physiol. Path. gén.* **16**, 212.
 — (1914, 2). *J. Physiol. Path. gén.* **16**, 386.
 — (1919). *Ann. Sci. Nat.* 10 Ser. **14**, 1.
 Turner (1931). *Bull. Miss. agric. Exp. Sta.* 160.
 Wellmann (1908). *Pflüg. Arch. ges. Physiol.* **121**, 508.
 Whitehorn (1921). *J. biol. Chem.* **45**, 449.
 Wilder (1929). *J. biol. Chem.* **81**, 65.