Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 16, *Numbers* 1 *and* 2, *January and April*, 1941.

Printed in the Union of South Africa by the
Government Printer, Pretoria.

A Note on the Influence of a Bloodmeal Ration on Porphyrin Excretion in Normal Bovines.

By G. C. S. ROETS, Section of Chemical Pathology, Onderstepoort.

In the course of experiments on the coproporphyrin excretion of normal cattle it was observed that animals receiving bloodmeal in their rations excreted increased amounts of porphyrins. Since the diagnosis of congenital porphyrinuria is based partly on an abnormally high porphyrin excretion via faeces and urine it is of importance to know to what an extent the porphyrin output in normal animals may be influenced by dietetic factors, both as regards the actual increase, if any, and the nature of the porphyrins excreted. In this paper the effect of feeding sterilised porphyrin-freed bloodmeal of known haematin content is recorded.

The relation of porphyrins and other closely related pyrrole pigments to haemoglobin formation or destruction has been studied [Fischer, H. (1931), Petek, A. J. and Minot, G. R. (1934), Petek, A. J. (1936), Robscheit-Robbins, F. S. and Whipple, G. H. (1930), Kohler, G.O., Elvehjem, C. A. and Hart, E. B. (1939), Hughes, J. H. and Latner, A. L. 1937)], but although cases of anaemia have been reported upon where dietetic pyrrole substances influenced the haemoglobin production, nobody has as yet succeeded in producing an anaemia due to a deficiency of porphyrin precursors.

Porphyrin which is closely related to haemin is present in normal erythrocytes and their precursors [Vannotti, A. (1937), Fischer, H. (1933), Roth, E. (1935), Vigliana, E. and Angeliere, C. (1934), Burmester, R. B. (1937), Seggel, K. A. (1937), Seggel, K.A. (1934), Watson, C. J. and Clarke, W. O. (1937)]. Grotepass, W. (1937) isolated protoporphyrin III from normal erythrocytes. V. d. Berg and Revers (1932) observed that the liver is capable of converting protoporphyrin into coproporphyrin.

The total amount of coproporphyrin extracted from 10,000 litres of normal human urine was found to be 200 mg. No protoporphyrin could be detected [Grotepass, W. (1939), Deusberg, R. (1938)]. It should, however, be kept in mind that protoporphyrin is excreted in the urine in cases of liver and bile system diseases [Boas, I. (1933)].

Improved methods made it possible to estimate quantitatively the coproporphyrin excretion in normal urine and faeces, thus providing an experimental basis for comparative studies [Dobriner, K., Strain, W. H. and Localio, S. A. (1937); Fourie, P. J. J. and Roets, G. C. S. (1939); Dobriner, K. (1937)].

Experimental.

The experimental animals were 5-year-old grade Sussex steers bred at the Ermelo Experimental Farm. They were fed a basic daily ration consisting of 4 Kg. crushed yellow maize, 500 gm. veld hay, and 500 gm. green barley; to this as occasion demanded 200 gm. sterilised bloodmeal was added. No porphyrin could be detected in 100 gm. of such bloodmeal. In the first batch of bloodmeal used the haematin concentration was equivalent to 11·24 gm. haemoglobin, whilst in the second batch it was equivalent to 19·9 gm. haemoglobin per 100 gm. bloodmeal. The haematin determinations were made by the method described by Roets (1940).

The metabolism stables used were those described by Rimington, Roets and Fourie (1938). The animals were allowed 3-day periods to accustom themselves to the new conditions before collections of faeces and urine were made for analytical purposes.

In the case of 5466 and 6043 bloodmeal feeding was stopped when collections were commenced. The collections of faeces and urine were made over a period of 14 days. The quantitative porphyrin determinations were made daily on the faeces, and every second day on an aliquot of the combined two-day urine of each animal. The urine and faeces of 6491 and 6498 were examined daily. Collections of and determinations on the faeces and urine of 6491, were made three days before and six days after bloodmeal feeding had been stopped. The examination of the urine and faeces of bovine 6498 was made one month after bloodmeal feeding was stopped, collections being made for 5 days. Bloodmeal was again given, and determinations made for a further 5 days. After a rest period of 5 days without bloodmeal the second batch of bloodmeal was fed for three days before collections were resumed. After the sixth day of these collections, the bloodmeal was again stopped and collections continued for 5 more days.

Faeces.—The faeces were collected and mixed and a 200 gm. sample extracted as described by Fourie and Roets (1939). Porphyrin estimations were made before and after the final 5 per cent. hydrochloric acid solution of porphyrins had been shaken with chloroform. A 200 gm. sample of faeces yielded 31 c.c. of porphyrin solution in 5 per cent hydrochloric acid. Before the chloroform extraction 2 c.c. of this solution had to be diluted up to 6 c.c. to match the standard of 1 mg. per 100 c.c.

$$\text{The concentration therefore} = \frac{31}{100} \times \frac{6}{2} \times 1000\ \gamma \text{ per } 200 \text{ gm.}$$

$$= 930\ \gamma \text{ per } 200 \text{ gm.}$$

This concentration will hence be called the *Total Porphyrin Concentration.*

The amount of faeces passed during this day was 2,870 gm., thus the amount of *Total Porphyrins*

$$= \frac{2870}{200} \times \cdot 93 \text{ mg.}$$

$$= 13 \cdot 81 \text{ mg.}$$

It is fully realised that values obtained in such a way cannot be regarded as exact, since the intensity of the overlapping 550 and 557 bands of copro- and protoporphyrins respectively are together measured against that of the 550 band of the coproporphyrin standard. However the figures thus obtained give reliable comparative values.

The remaining 29 c.c. of the porphyrin solution in 5 per cent. hydrochloric acid, was then transferred to a separatory funnel, half this amount of chloroform added, shaken thoroughly and the chloroform solution then run off. This process was repeated at least three times fresh chloroform being used each time. A final ether washing removed the chloroform, leaving a clear solution. Of this final solution 5 c.c. had to be diluted to 6 c.c. to match the standard. Thus the *coproporphyrin concentration*

$$= \frac{31}{100} \times \frac{6}{5} \times 1000 \ \gamma \text{ per 200 gm.}$$

$$= 372 \ \gamma \text{ per 200 gm.}$$

$\therefore$ *Total coproporphyrin* in this sample of faeces

$$= \frac{2870}{200} \times \cdot 372 \text{ mg.}$$

$$= 5 \cdot 524 \text{ mg.}$$

Urine (24 hour samples).—When the amount of 2,000 c.c. urine could not be obtained, the total amounts available were used for extractions. The ether extractions of the acidified urine sample was made by shaking the urine 3 times in succession in a separatory funnel with half the amount of ether using fresh ether for each shaking. The combined ether solution thus obtained from the urine sample is transferred to a separatory funnel, washed and extracted as described by Fourie and Roets (1939). No difference could be observed in the intensity of the spectrum of the final porphyrin solution in 5 per cent. hydrochloric acid before and after shaking with chloroform. Determinations were therefore made only after the chloroform shakings.

$\frac{\text{Coproporphyrin I}}{\text{Coproporphyrin III}}$ Ratio.

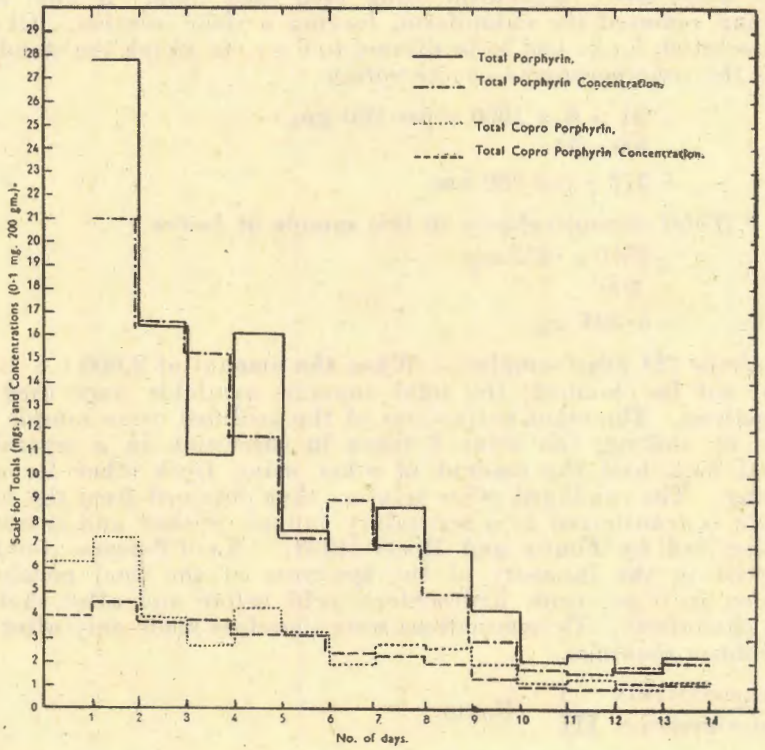
For the purpose of examining the relation of coproporphyrin I to coproporphyrin III in the urine and faeces of animals on rations with or without bloodmeal, the porphyrin fractions, after the determinations had been made, were accumulated. Collections from animals on a bloodmeal diet were commenced 3 days after bloodmeal was fed. The coproporphyrins from such samples were precipitated by neutralising with sodium hydroxide. Esterification, separation of the methyl esters and quantitative determinations were made as described by Rimington, Roets and Fourie (1938).

Results.

Faeces.—The results obtained on the faeces are diagramatically presented in Figures I, II, III and IV for animals 6043, 5466, 6491 and 6498 respectively. The relations of the values for (*a*) total porphyrin and (*b*) total coproporphyrin concentrations and (*c*) the total porphyrin and (*d*) the total coproporphyrin output for successive days are shown. For further details see Appendix Tables 1, 2 and 3.

The ratio of coproporphyrin I to coproporphyrin III in a sample accumulated from the faeces of 6491 and 6498 on rations free from bloodmeal was found to be 1 : 1·57, whilst the ratio in a sample accumulated from the faeces of the same animals on the ration containing the bloodmeal supplement was 1 : 1·4.

Fig. 1.—(6043) Faeces.

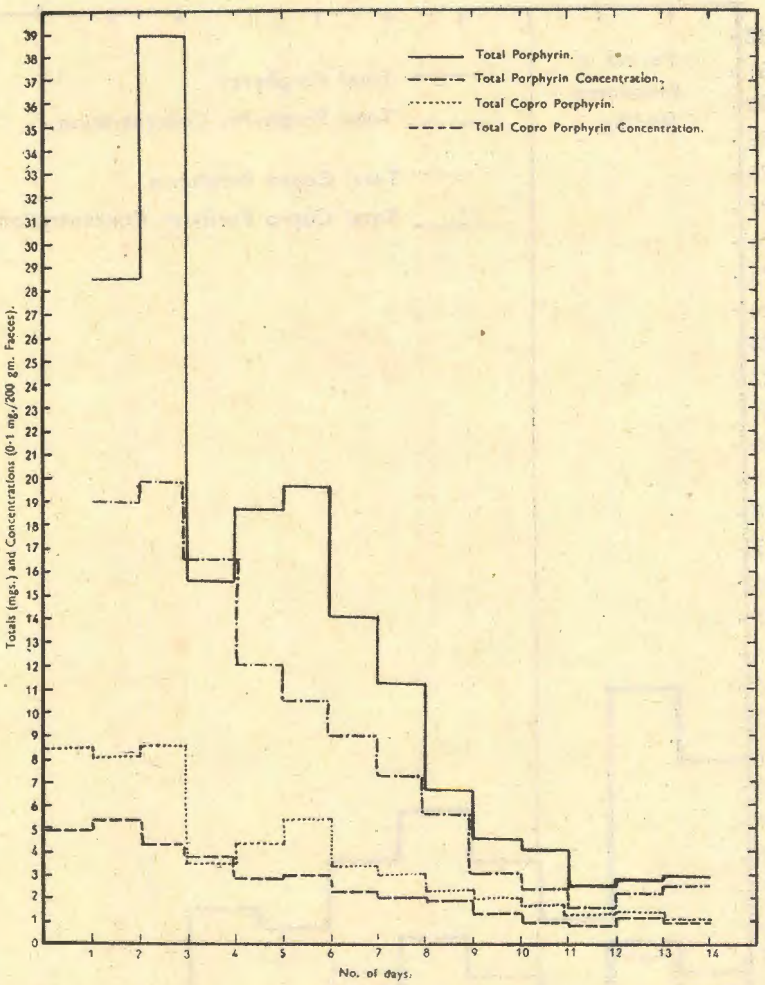


The recrystallised methyl esters obtained from these samples when mixed with pure materials did not show depressions of melting points.

To obtain an idea of the percentage of haematin of such sterilised bloodmeal digested, the faeces of 6498 were examined on 22.4.1940 and the concentration was found to be equivalent to 0·598 gm. haemoglobin per 100 gm. faeces. The total amount excreted for

that day was equivalent to 21·25 gm. haemoglobin. The daily amount fed was equivalent to 39·8 gm. haemoglobin, the amount digested thus being 18·45 gm. or approximately 46 per cent. haemoglobin for this animal on this particular day.

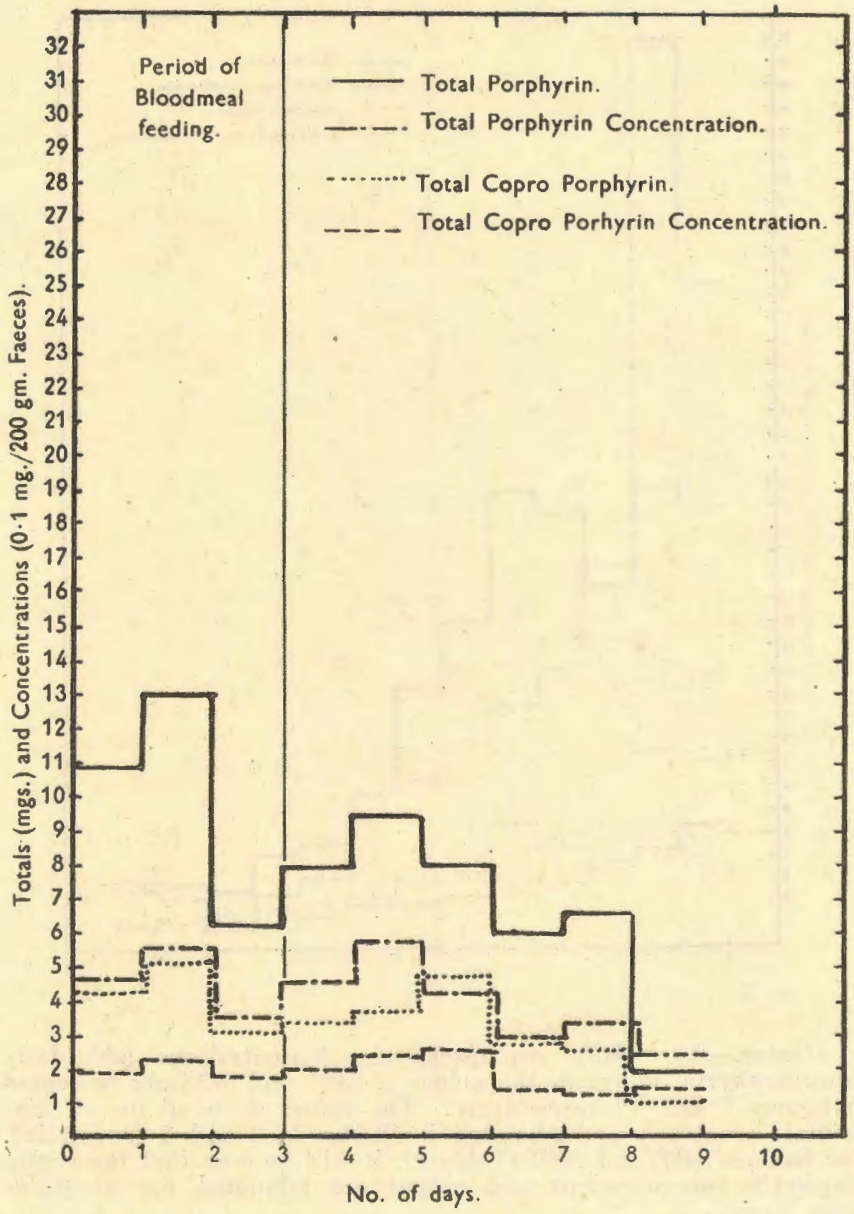
Fig. 2.—(5466) Faeces.



Urine.—The daily coproporphyrin concentrations and daily coproporphyrin output in the urines of 6491 and 6498 are presented in figures V and VI respectively. The values in detail for all four animals concerned are tabulated in Tables 1, 2 and 3 (appendix). For bovines 5466 and 6040 (Table 1), it will be seen that the coproporphyrin concentrations and output are tabulated for successive 2-day periods.

The ratio of coproporphyrin I to coproporphyrin III in a sample accumulated from the urines of 6491 and 6498, whilst on a ration of bloodmeal, was found to be 1 : 7·26. Unfortunately the amount collected from the urine of cattle fed on a bloodmeal free diet was insufficient for an analysis.

Fig. 3.—(6491) Faeces.



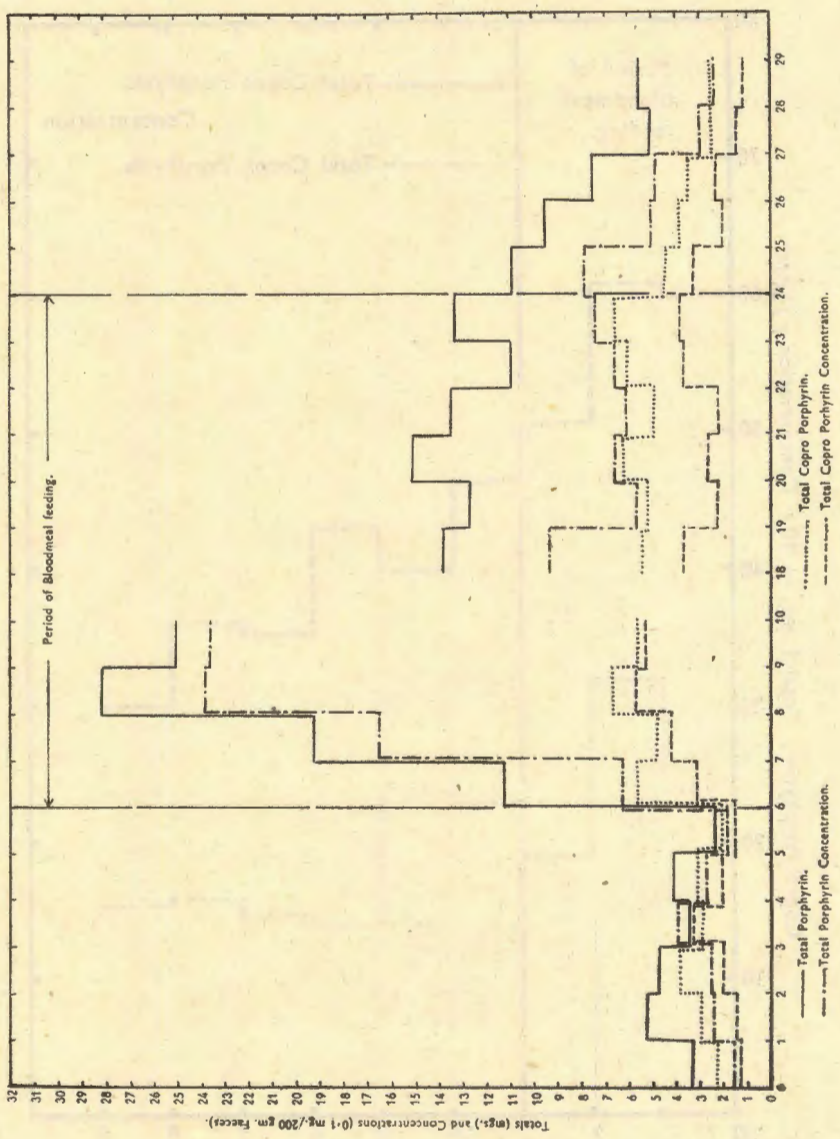
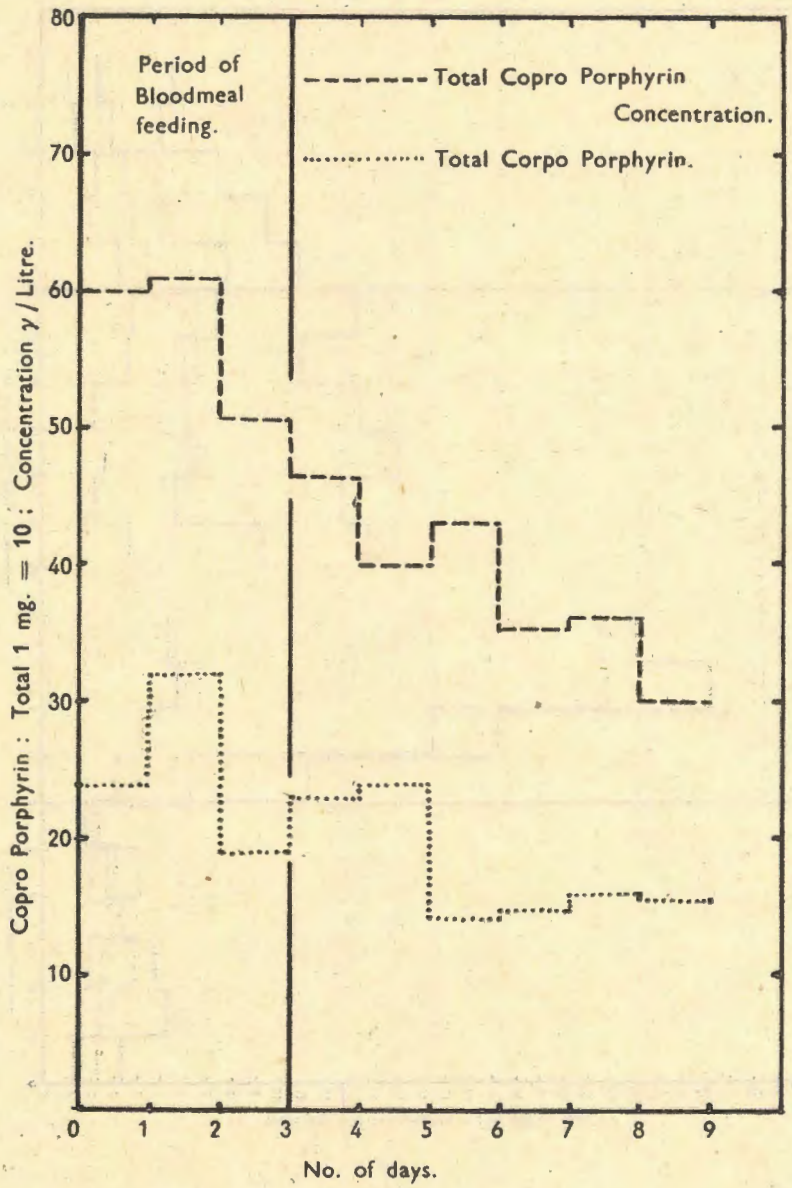


Fig. 4.—(6498) Faeces.

Fig. 5.—(6491) Urine.



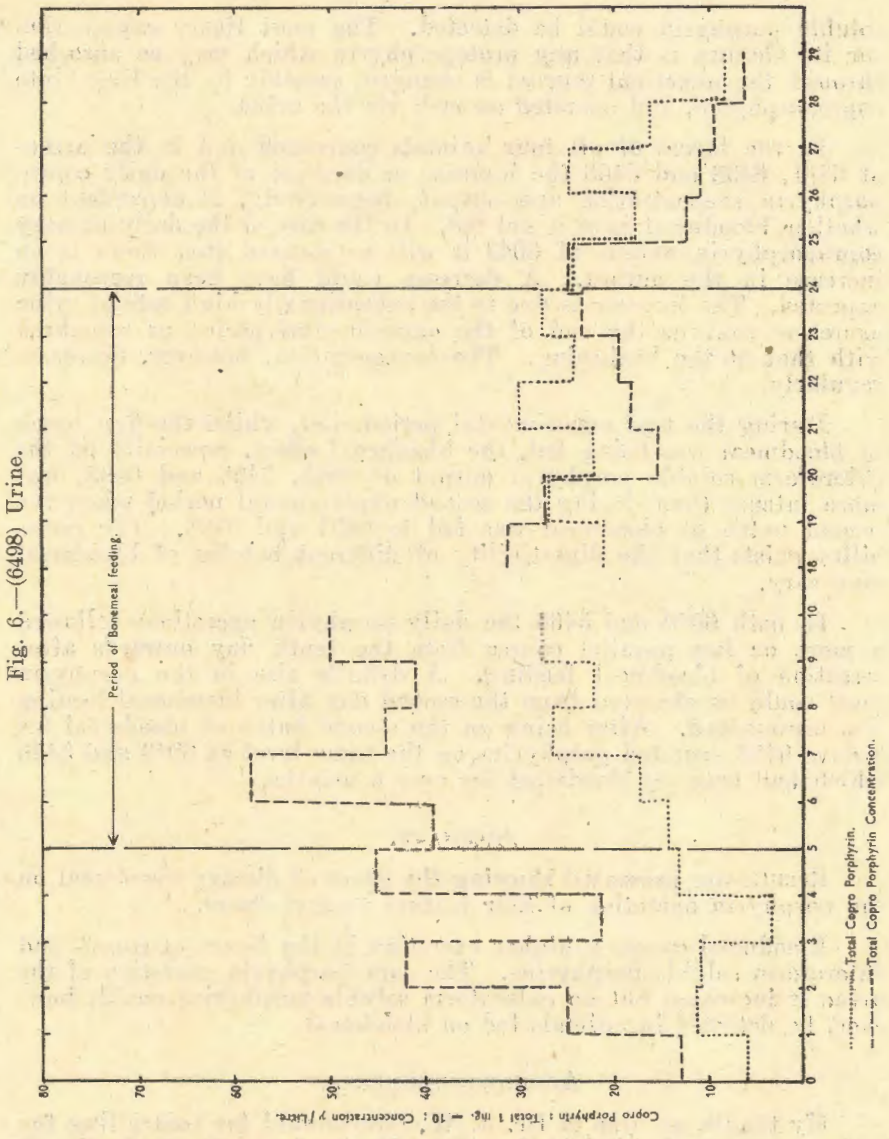


Fig. 6.—(6498) Urine.

Discussion.

On examination of the data it is obvious that the total porphyrin concentrations and output in the faeces promptly respond to dietary bloodmeal, the chloroform-soluble porphyrin being chiefly responsible for the increases noted. According to spectroscopic and methyl ester melting point examinations this porphyrin fraction probably consisted mainly of protoporphyrin (identifications will be fully reported on in a later communication). In the urines, however, no chloroform

soluble porphyrin could be detected. The most likely explanation for its absence is that any protoporphyrin which may be absorbed through the intestinal mucosa is changed, possibly by the liver, into coproporphyrin, and excreted as such via the urine.

In the faeces of all four animals concerned and in the urines of 6491, 6498 and 5466 the increase or decrease of the daily coproporphyrin concentration and output, respectively, is dependent on whether bloodmeal is or is not fed. In the case of the daily urinary coproporphyrin output of 6043 it will be noticed that there is an increase in the output. A decrease could have been reasonably expected. The increase is due to the exceptionally high rate of urine excretion towards the end of the experimental period as compared with that at the beginning. The concentration, however, decreases regularly.

During the first experimental period, i.e., whilst the first batch of bloodmeal was being fed, the bloodmeal effect, especially on the chloroform soluble porphyrin output of 6043, 5466 and 6498, was more intense than during the second experimental period when the second batch of bloodmeal was fed to 6491 and 6498. The possibility exists that the digestibility of different batches of bloodmeal may vary.

In both 6043 and 5466 the daily porphyrin excretions followed a more or less parallel course from the tenth day onwards after cessation of bloodmeal feeding. A definite rise in the porphyrin level could be observed from the second day after bloodmeal feeding was commenced. After being on the second batch of bloodmeal for 4 days 6498 excreted porphyrins on the same level as 6043 and 5466 which had been on bloodmeal for over 6 months.

Summary.

Results are presented showing the effect of dietary bloodmeal on the porphyrin excretion of four mature normal steers.

Bloodmeal causes a higher excretion in the faeces of copro- and chloroform soluble porphyrins. The coproporphyrin excretion of the urine is increased but no chloroform soluble porphyrins could, however, be detected in animals fed on bloodmeal.

Acknowledgements.

My thanks are due to Dr. J. W. Groenewald for controlling the rations. I am also indebted to Drs. H. Graf and P. J. J. Fourie for the interest they took in this work.

REFERENCES.

BOAS, I. (1933). Über das Vorkommen von Protoporphyrin im Harn. *Klin. Wochnschr.*, Vol. 12, pp. 589-591.

DEUSBERG, R. (1938). Über den Auf- und Abbau des Blutfarbstoffes. *Klin. Wochnschr.*, Vol. 17, p. 1353.

DOBRINER, K. (1937). Porphyrin excretion in faeces in normal and pathological conditions. *J. Biol. Chem.*, Vol. 120, pp. 115-127.

DOBRINER, K. (1937). Excretion of porphyrin by dogs. *Proc. Soc. Expt. Biol. Med.*, Vol. 36, p. 757.

DOBRINER, K., STRAIN, W. H., AND LOCALIO, S. A. (1937). Quantitative measurements of coproporphyrin and total coproporphyrin I excretion in normals. *Proc. Soc. Expt. Biol. Med.*, Vol. 36, pp. 752-754.

FISCHER, H. (1931). Haemin and relation of haemin to chlorophyll. *Z. Angew. Chem.*, Vol. 44, p. 617.

FOURIE, P. J. J., AND ROETS, G. C. S. (1939). Quantitative studies upon porphyrin excretion in bovine congenital porphyrinuria (Pink Tooth) No. 2. *Onderstepoort J.*, Vol. 13, Nos. 1 and 2, pp. 369-382.

GROTEPASS, W. (1938). Zur Kenntnis der Natürlichen Harnporphyrine. *Z. Physiol. Chem.*, Vol. 253, p. 276-281.

HAWKINS, W. B., ROBSCHEIT-ROBBINS, F. S., AND WHIPPLE, G. H. (1938). Haemoglobin production in anemia as influenced by the bile fistula. *Jnl. Exp. Med.*, Vol. 67, p. 89.

HUGHES, J. H., AND LATNER, A. L. (1936). Chlorophyll and haemoglobin regeneration after haemorrhage. *J. Physiol.*, Vol. 86, pp. 388-395.

HUGHES, J. H., AND LATNER, A. L. (1937). Aetioporphyrin and haemoglobin after haemorrhage. *J. Physiol.*, Vol. 89, pp. 403-406.

KELLER, C. J., UND SEGGEL, K. A. (1934). Über das Vorkommen fluorescierender Erythrocyten. I .Untersuchungen am normalen roten Blutbild. *Fol. Haemat.*, Vol. 52, pp. 241-249.

KOHLER, G. O., ELVEHJEM, C. A., AND HART, E. B. (1939). The relation of pyrrole-containing pigments to haemoglobin synthesis. *J. Biol. Chem.*, Vol. 128, p. 501.

PATEK, A. J., AND MINOT, G. R. (1934). Bile pigment and haemoglobin regeneration. The effect of bile pigment in cases of chronic hypochronic anemia. *Am. J. Med. Sc.*, Vol. 188, p. 206.

PATEK, A. J. (1936). Chlorophyll and regeneration of the blood. Effect of administration of chlorophyl derivatives to patients with chronic hypochronic anemia. *Arch. Int. Med.*, Vol 57, p. 73.

RIMINGTON, C., ROETS, G. C. S., AND FOURIE, P. J. J. (1938). Quantitative studies upon porphyrin excretion in bovine congenital porphyrinuria (Pink Tooth) No. 1. *Onderstepoort J.*, Vol. 10, No. 2, pp. 421-429.

ROETS, G. C. S. (1940). Chemical blood studies VIII. A rapid spectroscopic method for: (*a*) The quantitative determinition of haemoglobin in blood and (*b*) its application for the quantitative estimation of haemoglobin in milk, urine, serum or plasma and faeces. *Onderstepoort J.*, Vol. 14, pp. 451-458.

ROBSCHEIT-ROBBINS, F. S., AND WHIPPLE, G. H. (1930). Blood regeneration in severe anemia XIX. Influence of spinach, cabbage, onions and orange juice. *Am. J. Physiol.*, Vol. 92, pp. 400-407.

ROTH, E. (1935). Über zwei besondere Fälle von chronischer Porphyrie. *Deutsch. Arch. Klin. Med.*, Vol. 178, pp. 185-200.

SEGGEL, K. A. (1934). Über das Vorkommen fluorescierender Erythrocyten. II. Untersuchungen an Anämiefällen des Menschen. *Fol. Haemat.*, Vol. 52, p. 250.

VANNOTTI, A. (1937). Porphyrine und porphyrinkrankheiten. Berlin.

WATSON, C. J., AND CLARKE, W. O. (1937). The occurrence of protoporphyrin in the reticulocytes. *Proc. Soc. Expr. Med.*, Vol. 36, pp. 65-70.

APPENDIX.

TABLE 1.

No. of Animals.	Date.	Faeces.					Urine.			
		Amount in gm.	Coproporphyrin in γ per 200 gm.	Total Coproporphyrin in mg.	Total Porphyrins in γ per 200 gm.	Total Porphyrins in mg.	Amount.	Coproporphyrin in γ per 2,000 c.c.	Total Coproporphyrins in mg.	Water Intake in Litres.
5466.....	22–23/3/39	3030	538	8·151	1890	28·634	1440	—	—	8
	23–24/3/39	3930	436	8·567	1988	39·064	1300	143	·177	0
	24–25/3/39	1880	396	3·469	1650	15·51	1200			22
	25–26/3/39	3100	281	4·305	1200	18·6	2200	90	·144	0
	26–27/3/39	3750	288	5·4	1050	19·688	1000			0
	27–28/3/39	3140	213	3·344	900	14·13	935	74	·09	18
	28–29/3/39	3100	200	3·1	725	11·237	1500			0
	29–30/3/39	2350	188	2·303	560	6·86	1200	120	·140	4
	30–31/3/39	3050	131	1·998	300	4·575	1140			0
	31/3–1/4/39	3350	97	1·625	245	4·104	1050	100	·098	2
	1– 2/4/39	3150	88	1·386	156	2·457	800			18
	2– 3/4/39	2550	114	1·396	220	2·805	1700	81	·108	0
	3– 4/4/39	2350	93	1·093	250	2·938	980			0
	19–20/4/39	2850	121	1·724	225	3·206	940	72	·116	5
	20–21/4/39	4550	117	2·652	315	7·166	2240			—
6043.....	22–23/3/39	2650	558	7·394	2100	27·825	2730	—	—	3
	23–24/3/39	2050	375	3·844	1650	16·413	1500	165	·18	0
	24–25/3/39	1450	375	2·719	1513	10·969	740			22
	25–26/3/39	2750	314	4·318	1170	16·088	4470	78	·292	8
	26–27/3/39	2100	319	3·349	735	7·718	3020			4
	27–28/3/39	1550	243	1·883	900	6·975	820	82	·111	5·5
	28–29/3/39	2500	236	2·768	700	8·75	1880			16
	29–30/3/39	2850	188	2·679	490	6·983	7300	70	·313	0
	30–31/3/39	3000	125	1·875	285	4·275	1640			20
	31/3–1/4/39	2350	94	1·105	175	2·056	5300	39	·183	8
	1– 2/4/39	2900	84	1·218	168	2·436	4100			0
	2– 3/4/39	1950	100	0·975	180	1·755	720	58	·171	15·8
	3– 4/4/39	2300	110	1·265	190	2·185	5150			—
	19–20/4/39	2450	86	1·054	200	2·45	5800	46	·235	12
	20–21/4/39	2270	114	1·294	385	4·37	4400			

TABLE 2.

Bovine 6498.

Date.	Faeces.					Urine.				
	Amount in gm.	Coproporphyrin in γ per 200 gm.	Total Coproporphyrin in mg.	Total Porphyrins in γ per 200 gm.	Total Porphyrins in mg.	Amount in c.c.	Coproporphyrin in γ per 2,000 c.c.	Total Coproporphyrin in mg.	Total Coproporphyrin in Faeces and Urine in mg.	Water Intake in Litres.
27–28/3/40	3770	122	2·3	172	3·242	4700	26	0·061	2·361	7
28–29/3/40	4250	140	2·975	246	5·227	4720	50	0·118	3·113	11
29–30/3/40	3820	200	3·82	250	4·78	2620	84·6	0·111	3·931	14
30–31/3/40	1750	326	2·853	390	3·413	1700	43·8	0·037	2·89	14
31/3–1/4/40	3070	202	3·101	270	4·145	3000	90	0·135	3·236	14
1– 2/4/40	2580	158	2·038	180	2·322	3750	78	0·146	2·184	8
2– 3/4/40	3620	312	5·65	626	11·23	3080	116	0·179	5·829	16
3– 4/4/40	2330	412	4·8	1650	19·223	6120	80	0·269	5·069	12
4– 5/4/40	2370	568	6·73	2378	28·179	5500	82	0·226	6·956	8
5– 6/4/40	2120	538	5·702	2360	25·016	6020	100	0·301	6·003	26
16–17/4/40	2870	374	5·524	930	13·81	7000	62·5	0·219	5·743	18
17–18/4/40	4570	230	5·256	560	12·796	10140	54	0·274	5·530	22
18–19/4/40	4570	275	6·284	660	15·08	14350	31·25	0·224	6·508	20
19–20/4/40	4420	225	4·973	608	13·473	16000	37·5	0·3	5·273	24
20–21/4/40	3297	367	6·052	660	10·8834	12240	39·3	0·2405	6·2925	20
21–22/4/40	3570	370	6·6045	740	13·209	11800	46·9	0·277	6·8815	6
22–23/4/40	2780	325	4·5175	780	10·842	1000	50	0·25	4·7675	15
23–24/4/40	3800	210	3·99	500	9·5	15180	25	0·1798	4·1698	20
24–25/4/40	3050	236	3·599	495	7·549	22260	22	0·2448	3·8438	24
25–26/4/40	3370	150	2·5275	300	5·055	17020	19	0·1617	2·6892	18
26–27/4/40	4470	120	2·664	250	5·588	13500	12·5	0·0834	2·7474	26

TABLE 3.

Bovine 6491.

Date.	Faeces.					Urine.				
	Amount in gm.	Coproporphyrin in γ per 800 gm.	Total Coproporphyrin in mg.	Total Porphyrins in γ per 200 gm.	Total Porphyrins in mg.	Amount in c.c.	Coproporphyrin in γ per 2,000 c.c.	Total Coproporphyrin in mg.	Total Coproporphyrin in Faeces and Urine in mg.	Water Intake in Litres.
10–11/6/40	4780	184	4·398	460	10·994	6020	120	0·2412	4·6392	0
11–12/6/40	4710	222	5·228	555	13·07	5000	128	0·32	5·548	20
12–13/6/40	3580	175	3·133	350	6·265	3720	105	0·1953	3·1953	0
13–14/6/40	3530	195	3·442	450	7·943	5100	93	0·2372	3·6792	10
14–15/6/40	3330	228	3·796	570	9·491	6020	80	0·2408	4·0374	12
15–16/6/40	3830	252	4·826	420	8·043	3300	86	0·1419	4·9679	10
16–17/6/40	4180	138	2·884	288	6·0192	4200	71	0·1491	3·0335	10
17–18/6/40	4030	132	2·6598	330	6·6495	4500	72	0·162	2·8218	14
18–19/6/40	1630	144	1·1736	240	1·956	4920	60	0·1576	1·3312	—