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Quantitative Studies upon Porphyrin Excretion in Bovine Congenital Porphyrinuria (Pink Tooth) No. 2.

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THE first known living cases of bovine congenital porphyrinuria were described by Fourie (1936) from the clinical and pathological, and by Rimington (1936), Rimington and Fourie (1937), Rimington and Roets (1937) from the bio-chemical point of view. Rimington, Roets and Fourie (1938) presented certain details concerning the quantitative excretion of porphyrins in these cases, but at that time the authors felt that the data were incomplete and the results reported in this paper are intended to be supplementary to those previously presented.

Fischer, quoted by Schumm (1919), found ·17 gm. to ·5 gm. of porphyrin pigments daily in the urine of Petry when he made the determinations during different months of the year (November to February). Schumm (1919) examined the urine of Petry during the summer and during the winter and found more or less the same amounts (circa 0·4 gm.) on both occasions.

Beyond this very little seems to be known concerning the quantitative excretoin of porphyrins in congenital porphyrinuria in man and animals. Recently, however, a good deal of work is reported concerning the quantitative determination of porphyrins in the case of normal humans as well as in cases of man and animals suffering from various diseases, e.g. Dobriner (1937), Brugsch (1938), Rimington (1938), Grotepass (1938), Mertens (1937), and Roets (1938). In these papers notable advances are recorded concerning the methods employed in order to determine quantitatively the porphyrins excreted.

In this paper the authors intend to record results concerning the quantitative porphyrin excretion during different times of the year by bovines suffering from congenital porphyrinuria (Pink

Tooth), and at the same time data are presented concerning the relative porphyrin excretion in bovine congenital porphyrinuria in a completely normal bovine and in apparently* normal bovines.

Details of Experiments.

The animals were maintained in metabolism stables on a daily ration of 5 pounds veld hay in the morning and 8 pounds crushed maize with 2 pounds of green food in the afternoon. The animals were watered twice daily and the amount of water consumed was carefully measured.

The amount of faeces passed and the amount of urine excreted were carefully weighed and measured every 24 hours. The animals were usually under observation for a period of 14 days, but determinations were not recorded for the first 24-48 hours of the experiment, as the animals were sometimes a little bit upset when they were confined to the metabolism stables, resulting in some cases in diarrhoea. During this 24-48 hour period they were therefore allowed to settle down to the conditions of watering, feeding, etc.

Daily quantitative porphyrin determinations of faeces and urine were made in the case of the known pink tooth animals 7016, 7017 (see Fourie, 1936) in the same way as this was done by Rimington, Roets and Fourie (1938).

On account of the low level of porphyrin excretion in the normal animal 6384 and in the apparently normal animals 7022, 7393 and 7356 a slightly modified method of dealing with the faeces and urine was made use of. Before giving details of the method in which the faeces and urine were actually handled, it is perhaps just as well to give a brief history of these animals.

No. 6384 is a red heifer entirely unrelated to the porphyrin animals, but the bull 7015 (see Fourie, 1936) which is known to transmit the condition is being mated to her, in an attempt to produce further porphyrin carriers for the study of the genetics of congenital porphyrinuria.

No. 7393 is a carrier, being the daughter of a porphyrin cow.

No. 7022 is a daughter of Bull 7015 and thus possibly a carrier—she has, however already produced 2 calves from matings to her own father, but both calves are clinically normal.

No. 7356 is a heifer and one of the two calves of 7022.

The last three animals (7393, 7022, and 7356) are therefore listed as apparently normal as one certainly and two possibly may be carriers of the recessive character.

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* Apparently normal is here used for animals which in some cases are actually known to be carriers of the anomaly, and in other cases for animals which may be carriers, since they are daughters of the bull 7015 (see Fourie, 1937) which is known to be a carrier of the recessive character. A paper concerning the hereditary nature of the condition as a recessive character will be published by one of us (Fourie) at a later date.

In addition to these three animals in this group, a further animal was added, viz. No. 7597. He is a bull which is showing very marked photosensitization on exposure to the sun. His teeth are only slightly discoloured if at all and porphyrin excretion in the faeces and urine is on a low level. This animal, however, has a remote common ancestry to bull 7015 and is at present being regarded as a very suspicious but atypical case of congenital bovine porphyrinuria. Confirmation of this is being awaited, pending the results of certain breeding experiments already commenced.

Method of Dealing with Urine and Faeces of Normal and Apparently Normal Animals, etc.

Urine.

A representative sample of 500 c.c. of urine from each animal was *collected* at the end of each 24 hour period. It was stored in the ice chest and every 24 hours an aliquot of 500 c.c. of that day was added to that collected on previous days, until at the end of four days 2,000 c.c. of urine was available for examination from each animal. Acetic acid was then added up to a concentration of 5 per cent., and the solution extracted in a big Kutcher-Steudal continuous extraction apparatus for 24 hours. The ethereal solution was thoroughly washed in a separatory funnel with water containing potassium acetate, in order to remove the acetic acid and any soluble urinary pigments. The water washings were, however, rewashed with fresh ether to safeguard against any possible porphyrin loss. Any porphyrin so obtained in ether solution was then added to the main ether solution containing porphyrin so that the final ether solution usually had a volume of approximately 750 c.c. From this solution the porphyrins were transferred to acid solution by repeatedly adding to the former 10 to 15 c.c. of 5 per cent. HCl and shaking thoroughly. Negative fluorescence in ultra violet light indicates when the end point has been reached. The entire acid solution about 50 c.c. in volume, was transferred to a 100 c.c. separatory funnel, potassium acetate added as before, the porphyrins again shaken into ethereal solution and the water washings referred to above repeated. The porphyrins from this final solution, which was approximately 30 c.c. in volume was shaken into 5 to 10 c.c. of 5 per cent. HCl, and the intensity of the 550 band compared spectroscopically with a porphyrin standard of known value in the usual way.

Because of the small amount of porphyrin present in these animals, two of them (one 7597 the suspected porphyrin sufferer and the other 6384, completely normal) were used for further investigation concerning the presence of uroporphyrin, by adding acetic acid to their ether soluble porphyrin extracted urine to a concentration of 5 per cent. and filtering through alumina absorption columns, but no uroporphyrin could be detected by eluting with dilute alkali.*

* This method was devised and employed by one of us (Roets) in June (1938). Since this article was written up in November (1938) Dobriner and Rhoads (1938) independently used practically the same method to determine urinary coproporphyrin in the urine of man.

Faeces.

50 gm. of a representative sample of faeces from each animal was collected daily in acetic acid. This was kept in the dark and after 4 days the combined 200 gm. from each animal was extracted in the usual way with acetic acid and ether. The porphyrin was transferred to 5-20 c.c. of 5 per cent. HCl (depending on the porphyrin content). This solution was shaken with chloroform to remove soluble pigments and the copro-porphyrins determined.

The handling and sampling of the faeces and urine were otherwise carried out as described by Rimington, Roets and Fourie (1938).

Results Obtained.

Faeces and urine from the two pink tooth animals 7017 and 7018 were examined from 9/11/37 to 19/11/37 and again from 14/2/38 to 24/2/38. That from the third pink tooth animal 7016 was examined from 8/12/37 to 23/12/37. The results are recorded in tables 1, 2 and 3.

In Figs. I, II and III the daily total porphyrin, daily total copro-porphyrin and the daily total uroporphyrin, with corresponding averages for the three animals 7017, 7018 and 7016 are graphically presented.

The I and III series isomers of coproporphyrin were separated and quantitatively determined in urine samples of 7017, 7018, and in faeces samples of 7017, 7018, 7022 (apparently normal, possibly a carrier) and 7597 (suspected pink tooth bull) (Table 4) using the method described by Rimington, Roets and Fourie (1938). A portion of urine and of faeces excreted during a 24-hour period was taken for this purpose.

Analysing the figures presented in Tables 1, 2 and 3 there does not appear to be a consistent relationship between the daily porphyrin excretion and total daily faeces or urine output.

In the case of animal 7017, in which the greatest porphyrin excretion is recorded there was, after an initial rise in porphyrin excretion, a steady decline for the first few days after the animal was placed in the metabolism stable (admittedly under completely different environmental conditions than the animals were used to). Thereafter and until the completion of the ten day period the porphyrin excretion steadily increased, this being mainly due to an increase in the copro-porphyrin, as the uroporphyrin fluctuated irregularly during that time. This examination occurred during the early summer.

When the animal was again examined for ten days during the late summer, there was again an initial porphyrin decrease, but this time more regularly progressive than the previous time, in spite of a somewhat marked increase in the uroporphyrin at times. Subsequently, however, the porphyrin fluctuation was rather irregular.

In the other two animals (7018 and 7016) the porphyrin excretion fluctuated widely and irregularly from day to day.

TABLE 1.

(Bovine 7017.)

Date.	FAECES.			URINE.					Total Coproporphyrin in gm.	Total Uro- + Coproporphyrin in gm.	Water Intake in Litres.
	Weight in gm.	Coproporphyrin.		Volume in c.c.	Coproporphyrin.		Uroporphyrin.				
		Mg. per 100 gm.	Total in gm.		Mg. Per 100 cc.	Total in gm.	Mg. per 100 c.c.	Total in gm.			
9–10/11/37.....	7,410	8·0	0·5928	2,860	3·25	0·093	2·667	0·6763	0·6858	0·7621	11·5
10–11/11/37.....	8,750	9·6	0·8400	3,580	1·925	0·0689	1·867	0·0668	0·9089	0·9757	16·5
11–12/11/37.....	6,760	7·2	0·4867	4,825	·733	0·0354	1·333	0·0643	0·5221	0·5864	19·5
12–13/11/37.....	7,260	5·688	0·4129	2,190	2·167	0·0475	2·133	0·0467	0·4604	0·5071	14·5
13–14/11/37.....	6,810	8·125	0·5533	1,970	4·875	0·0960	2·667	0·0525	0·6493	0·7018	16·5
14–15/11/37.....	7,010	8·75	0·6134	3,705	4·5	0·1667	2·4	0·0889	0·7798	0·8687	17·75
15–16/11/37.....	6,090	12·0	0·7308	5,190	1·333	0·0692	1·667	0·0865	0·8000	0·8865	16·0
16–17/11/37.....	7,360	11·5	0·8464	4,150	2·75	0·1141	1·8	0·0775	0·9605	1·0380	20·0
17–18/11/37.....	6,310	14·25	0·8972	5,960	1·75	0·1043	2·0	0·1192	1·0035	1·1247	20·0
18–19/11/37.....	6,440	15·0	0·9660	4,560	2·333	0·1064	2·233	0·1034	1·0724	1·1758	2·75
AVERAGE....	7,020	10·011	0·6944	3,899	2·562	0·0915	2·077	0·0782	0·7843	0·8627	15·5
14–15/ 2/38.....	5,440	30·0	1·6320	1,445	5·5	0·0795	9·0	0·1301	1·7115	1·8416	16
15–16/ 2/38.....	6,820	20·0	1·3640	6,560	2·2	0·1443	3·75	0·2460	1·5083	1·7543	16·8
16–17/ 2/38.....	8,070	17·5	1·4123	2,310	6·0	0·1386	5·25	0·1213	1·5509	1·6722	6·0
17–18/ 2/38.....	6,670	18·0	1·2006	4,400	2·8	0·1232	4·5	0·1980	1·3238	1·5218	14·6
18–19/ 2/38.....	7,520	14·0	1·0528	4,830	2·8	0·1352	3·2	0·1546	1·1880	1·3426	4·4
19–20/ 2/38.....	8,980	16·0	1·4368	3,290	5·4	0·1777	4·5	0·1481	1·6145	1·7626	14·4
20–21/ 2/38.....	11,520	14·0	1·6128	2,680	5·25	0·1407	4·5	0·1206	1·7535	1·8741	14·4
21–22/ 2/38.....	9,070	12·0	1·0884	—	—	—	—	—	—	—	12·2
22–23/ 2/38.....	8,470	13·75	1·1646	2,600	4·5	0·1170	4·0	0·1040	1·2816	1·3856	8·0
23–24/ 2/38.....	9,220	16·25	1·4983	2,570	5·5	0·1414	4·2	0·1079	1·6397	1·7476	9·0
AVERAGE....	8,178	17·15	1·3463	3,409	4·217	0·1331	4·77	0·1478	1·5080	1·6558	11·58

TABLE 2.

(Bovine 7018.)

Date.	FAECES.			URINE.					Total Coproporphyrin in gm.	Total Uro- + Coproporphyrin in gm.	Water Intake in Litres.
	Weight in gm.	Coproporphyrin.		Volume in c.c.	Coproporphyrin.		Uroporphyrin.				
		Mg. per 100 gm.	Total in gm.		Mg. Per 100 cc.	Total in gm.	Mg. per 100 c.c.	Total in gm.			
9–10/11/37.....	8,810	3·57	0·3179	2,640	0·714	0·0189	1·143	0·0328	0·3368	0·3696	15·75
10–11/11/37.....	10,310	3·8	0·3918	5,270	0·5	0·0264	0·914	0·0482	0·4182	0·4664	17·0
11–12/11/37.....	8,710	1·95	0·1698	4·730	0·429	0·0203	1·143	0·0541	0·1901	0·2442	15·0
12–13/11/37.....	8,610	1·552	0·1336	4,000	0·476	0·0190	1·142	0·0457	0·1526	0·1983	17·75
13–14/11/37.....	8,510	1·8	0·1532	3,235	0·429	0·0130	1·286	0·0416	0·1662	0·2078	21·75
14–15/11/37.....	7,890	3·2	0·2525	3,900	0·771	0·0301	1·143	0·0474	0·2826	0·3300	20·75
15–16/11/37.....	5,740	3·0	0·1722	5,400	1·071	0·0536	1·143	0·0617	0·2258	0·2875	16·25
16–17/11/37.....	6,310	4·063	0·2564	7,160	0·686	0·0491	1·143	0·0818	0·3055	0·3873	19·75
17–18/11/37.....	5,210	3·125	0·1943	9,110	0·714	0·0650	0·914	0·0833	0·2593	0·3426	18·5
18–19/11/37.....	6,360	8·5	0·5406	8,930	0·643	0·0574	0·914	0·0816	0·5980	0·6796	9·0
AVERAGE....	7,646	3·446	0·2582	5,438	0·643	0·0353	1·089	0·0578	0·2935	0·3513	17·15
14–15/ 2/38.....	5,380	15·0	0·8070	8,440	0·594	0·0501	0·686	0·0579	0·8571	0·9150	29·2
15–16/ 2/38.....	7,850	10·0	0·7850	10,040	0·557	0·0559	0·937	0·0941	0·8409	0·9350	15·8
16–17/ 2/38.....	6,300	11·0	0·6930	7,130	0·857	0·0611	0·914	0·0652	0·7541	0·8193	14·4
17–18/ 2/38.....	5,790	12·0	0·6948	16,960	0·245	0·0416	0·353	0·0599	0·7364	0·7963	25·0
18–19/ 2/38.....	8,775	9·5	0·8336	14,380	0·274	0·0394	0·686	0·0986	0·8730	0·9716	14·4
19–20/ 2/38.....	7,970	8·0	0·6576	8,300	0·68	0·0564	0·914	0·0759	0·7140	0·7899	26·0
20–21/ 2/38.....	9,980	8·0	0·7984	z,920	1·114	0·0771	1·143	0·0791	0·8755	0·9546	22·0
21–22/ 2/38.....	8,100	9·5	0·7695	6,580	0·857	0·0564	1·143	0·0752	0·8259	0·9011	16·8
22–23/ 2/38.....	9,670	7·5	0·7253	6,660	0·857	0·0571	1·32	0·0879	0·7824	0·8703	18·0
23–24/ 2/38.....	9,200	7·0	0·6440	7,310	0·743	0·0543	0·875	0·0620	0·6983	0·7603	17·0
AVERAGE....	7,902	9·75	0·7408	9,272	0·678	0·0549	0·897	0·0756	0·7957	0·8713	19·9

TABLE 3.

(Bovine 7016.)

Date.	FAECES.			URINE.					Total Coproporphyrin in gm.	Total Uro- + Coproporphyrin in gm.	Water Intake in Litres.
	Weight in gm.	Coproporphyrin.		Volume in c.c.	Coproporphyrin.		Uroporphyrin.				
		Mg. per 100 gm.	Total in gm.		Mg. Per 100 cc.	Total in gm.	Mg. per 100 c.c.	Total in gm.			
8– 9/12/37.....	7,860	1·563	0·1229	2,500	0·767	0·0192	1·467	0·0367	0·1421	0·1788	—
9–10/12/37.....	6,910	3·8	0·2626	3,600	0·686	0·0247	1·143	0·0411	0·2873	0·3284	14·25
10–11/12/37.....	6,800	3·75	0·2550	4,620	0·557	0·0257	1·093	0·0505	0·2807	0·3312	17·75
11–12/12/37.....	7,460	3·6	0·2686	4,320	0·893	0·0386	1·143	0·0494	0·3072	0·3566	16·0
12–13/12/37.....	6,740	2·6	0·1752	5,880	0·513	0·0302	0·8	0·0470	0·2054	0·2524	9·9
13–14/12/37.....	7,410	3·0	0·2223	3,340	0·514	0·0172	1·029	0·0344	0·2395	0·2739	20·0
14–15/12/37.....	10,160	2·7	0·2743	4,400	0·32	0·0141	0·971	0·0427	0·2884	0·3311	16·25
16–17/12/37.....	6,260	3·3	0·2066	3,180	0·571	0·0182	1·6	0·0509	0·2248	0·2757	—
17–18/12/37.....	6,260	3·2	0·2000	4,960	0·374	0·0186	1·016	0·0504	0·2186	0·2690	24
18–19/12/37.....	8,610	3·4	0·2927	6,380	0·314	0·0200	—	—	0·3127	—	21·2
19–20/12/37.....	11,260	2·325	0·2618	4,035	0·42	0·0169	0·914	0·0369	0·2787	0·3156	19·5
20–21/12/37.....	6,940	3·14	0·2186	5,200	0·334	0·0174	0·824	0·0428	0·2360	0·2788	12·0
21–22/12/37.....	8,940	1·95	0·1743	4,000	0·33	0·0132	0·914	0·0366	0·1875	0·2241	20·5
22–23/12/37.....	9,360	1·06	0·0992	3,660	0·238	0·0087	0·857	0·0314	0·1079	0·1393	18·2
AVERAGE....	7,926	2·813	0·2167	4,191	0·488	0·0202	1·059	0·0424	0·2655	0·2888	16·12

FIG. I.—Bovine 7017.

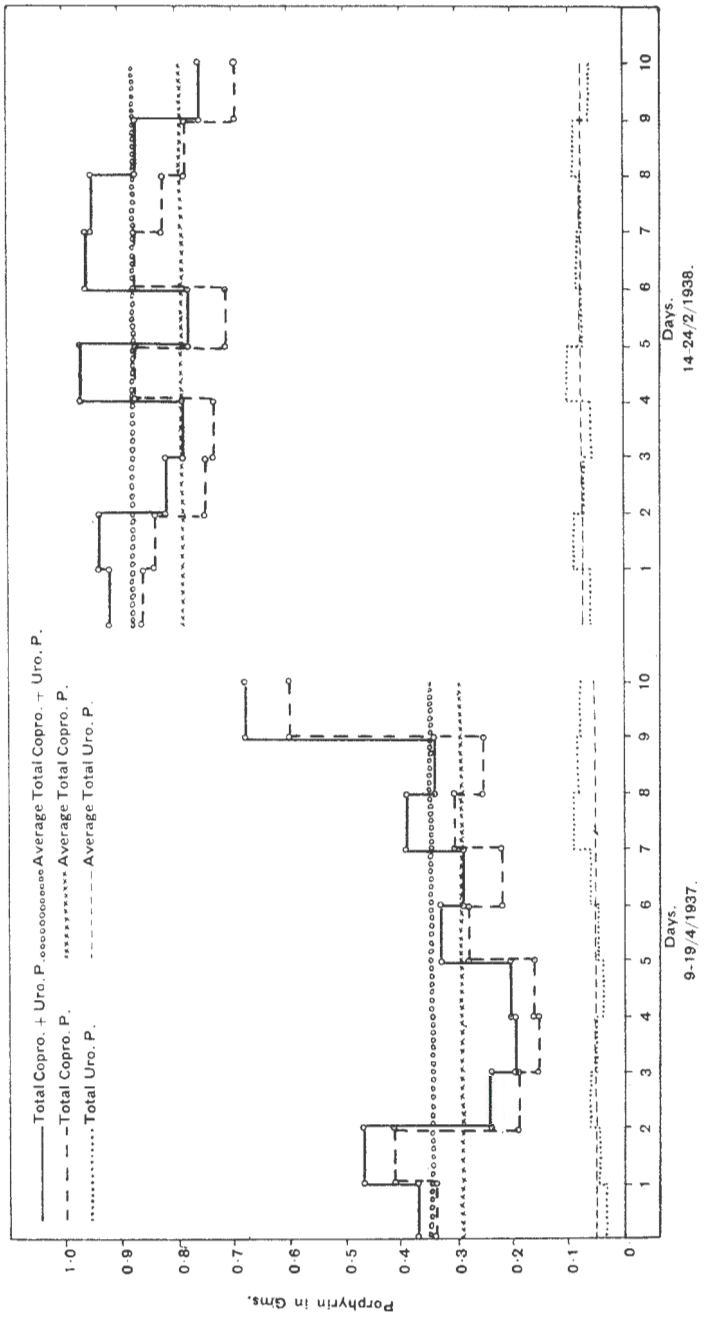

FIG. III.—Bovine 7016.

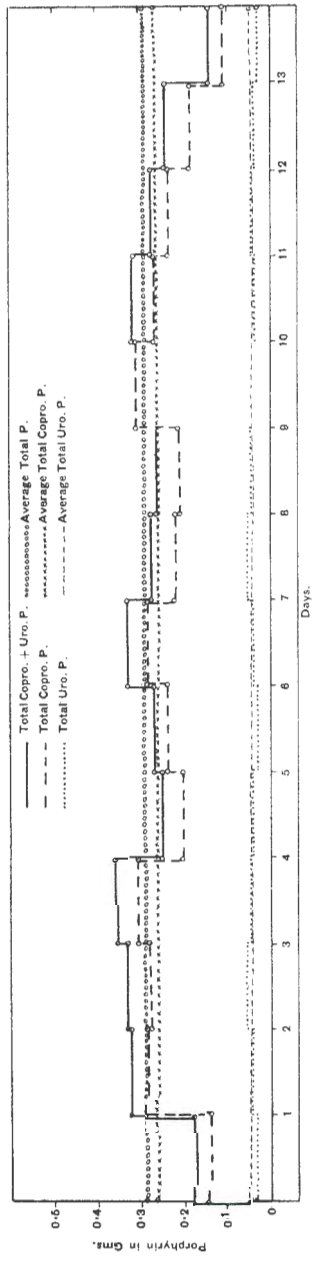

FIG. II.—Bovine 7018.

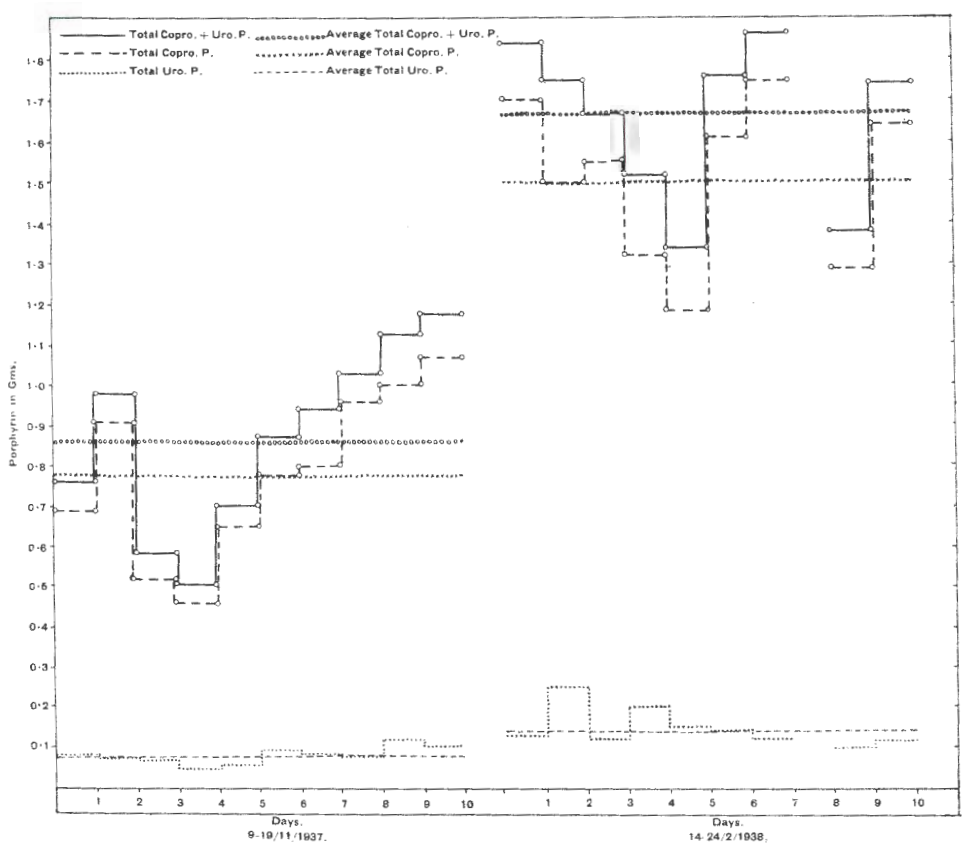

TABLE 4.

Ratio Copro I : Copro III.

No. of Animal.	Date.	Material.	Total Copro. in mg.	Copro. I in mg.	Copro. III in mg.	Ratio of Copro. I : III.
7017	18–19/11/37.	Urine.....	52·5	45·0	2·25	1 : 0·05
7018	18–19/11/37.	Urine.....	18·75	15·0	2·52	1 : 0·167
7017	23–24/ 2/38.	Faeces.....	41·0	37·5	1·68	1 : 0·04
7018	23–24/ 2/38.	Faeces.....	22·5	20·0	0·765	1 : 0·038
7022	24–25/ 6/38.	Faeces.....	—	0·105	0·065	1 : 0·619
7597	24–25/ 6/38.	Faeces.....	—	0·238	0·30	1 : 1·261

It is perhaps worthy of note that both the animals 7017 and 7018, which were always under observation at the same time, excreted porphyrin at a much higher level when examined during the late summer than was the case during the early summer. It is not considered likely that photosensitization may have been the cause of this, as the animals were only exposed for a very short time to the sun, when they were driven from one stable to the other. That the change in the season may have been a factor, cannot be definitely stated. However, it seems probable that seasonal variation was responsible for the increased porphyrin excretion during the late summer, since a change in food was certainly not a factor, as the two animals under conditions of stabling were given the same food on both occasions.

This higher level of porphyrin excretion in animal 7017 during the late summer was not so much due to a greater urine volume as to a greater porphyrin concentration, whereas in the case of 7018, the concentration of porphyrin in the urine was more or less the same during early spring, but as the total volume of urine was markedly increased (average daily output early spring = 5,438 c.c., average daily output late summer = 9,272 c.c.) the higher uro-porphyrin excretion was definitely due to this greater total urine output. However, in the case of the faeces of both animals the concentration was definitely higher during late summer than during the early spring, whilst the faeces weight remained at more or less the same level on both occasions. In order to eliminate the possibility that an experimental error may have been responsible for the higher values obtained during the late summer, new standards were made up and determinations were made in duplicate, when it was found that the results corresponded in both instances. Whether this seasonal variation in the porphyrin excretion can satisfactorily explain the uneven distribution of the porphyrins in bones of affected animals as described by Fourie (1936), is a point which cannot at the moment be definitely settled from the available information.

In working out the ratio of total to copro-to uro-porphyrin excreted from the daily averages (see Table 5) during the periods of 10 to 14 days the figures obtained are strikingly constant viz. approximately 10:9:1 for the three animals 7016, 7017 and 7018. There is however a deviation from the constant in the case of animal 7018, during the period 9/11/38 to 19/11/38, when the ratio is approximately 10:8:2. This deviation is due to the exceptionally large volume of urine which was excreted by this animal at that time as compared with the volume of urine excreted by this animal subsequently, and as compared with the amounts excreted by the other two animals.

TABLE 5.

No. of Animal.	Date.	Daily Average of Total Porphyrin in gm.	Daily Average Total Coproporphyrin in gm.	Daily Average Total Uroporphyrin in gm.	Ratio of— Total.	Copro.	Uro.
7017	14-24/ 2/38.....	1·6558	1·5080	0·1478	10	9·1	0·9
7017	9–19/11/37.....	0·8627	0·7841	0·0782	10	9·1	0·9
7018	14-24/ 2/38.....	0·8713	0·7957	0·0756	10	9·1	0·8
7018	9-19/11/38.....	0·3513	0·2935	0·0578	10	8·4	1·6
7016	8-23/12/37.....	0·2888	0·2655	0·0424	10	9·2	1·46

The average coproporphyrin concentration in the urine of the normal animal 6384, which is not in any way related to the congenital porphyrinuric animals, over a period of 8 days is ·056 mg. per 2,000 c.c. of urine. This is a little higher than the concentration in normal human urine as determined by Grotepass (1938), who found the concentration to be ·02 mg. per 1,000 c.c. in 10,000 litres of urine. In man the total average daily coproporphyrin excretion over 9-day periods is 306 to 376 micro-grams (Dobriner, 1937).

The figures obtained for the normal animal 6384 over an 8-day period are: (*a*) total daily average coproporphyrin excreted in the urine—·056 mg. per 2,000 c.c. and (*b*) the total daily average coproporphyrin excreted in the faeces—·056 mg. per 100 mg. When the excreted porphyrin of the apparently normal animals Nos. 7022 and 7393 (both being related to congenital porphyrinuria animals) is compared with that of the normal animal 6384 (see Table 6) it will be seen that the concentrations of coproporphyrin both in urine and faeces are slightly higher viz.:—

7022—urine, ·066 mg. per 2,000 c.c.
faeces, ·11 mg. per 100 gm.

and 7393—urine, ·069 mg. per 2,000 c.c.
faeces, ·105 mg. per 100 gm.

TABLE 6.

Showing concentration of coproporphyrin in faeces and urine of one normal animal (6384), *of three apparently normal animals* (7022, 7356, 7393) *and of one suspected case of Pink Tooth* (7597).

No. of Animal.	Date.	Faeces.		Urine.		
		Weight in gm.	Coproporphyrin mg. per 100 gm.	Volume in c.c.	Coproporphyrin mg. in 2,000 c.c.	Water Intake in Litres.
7022.....	16–20/6/38	40,880	0·102	7,790	0·058	35·8
	20–25/6/38	33,200	0·116	7,790	0·072	37·6
Daily Average...	16–25/6/38	8,231	0·110	1,731	0·066	8·2
7597.....	16–20/6/38	26,140	0·144	11,575	0·147	33·4
	20–25/6/38	41,420	0·15	16,770	0·08	47·2
Daily Average...	16–25/6/38	7,537	0·147	3,149	0·11	8·96
6384.....	11–15/7/38	26,430	0·053	5,330	0·06	21·6
	15–19/7/38	19,050	0·058	13,615	0·051	29·4
Daily Average...	11–19/7/38	5,685	0·056	2,368	0·056	6·4
7393.....	11–15/7/38	28,230	0·111	7,322	0·053	24·6
	15–19/7/38	26,300	0·098	11,500	0·083	43·0
Daily Average...	11–19/7/38	6,059	0·105	2,353	0·068	8·5
7356.....	13–19/7/38	18,640	0·058	10,620	0·073	40·0
Daily Average...	—	3,107	0·058	1,770	0·073	6·7

Whether these values also fall within the limits of normality is not known at the moment.

Animals 7022, 7393, 7356 and 6384 were originally included in these experiments as a group of normal controls but when the porphyrin values of three of them were found to be higher than the completely normal animal 6384 which is not in any way related to porphyrinuric animals, we began to doubt if their porphyrin values can be regarded as normal. This is most unfortunate as we now have values for only one normal animal (6384). However, we hope soon to have results of at least 8 normal animals, which will be examined at different times of the year and it is only when these figures are available that we shall be in a position to state what the quantitative porphyrin excretion in the normal bovine is.

Animal 7356 (known carrier) was also examined but as this animal had rather a profuse diarrhoea at the time of the examination, it is not used for purposes of comparison, as the digestive disturbance may possibly influence the values obtained.

In the case of the suspected porphyrinuric bull 7597 showing marked and continuous photosensitization the porphyrin excretion in faeces and urine is at a higher level than in the normal animal 6384 but very much less than in the undoubted pink tooth cases.

The ratio of coproporphyrin I to coproporphyrin III (see Table 4) in faeces and urine varies from animal to animal. In man Grotepass (1938) puts the average ratio of the I to the III series in normal human urine as 96 : 87.

Calculating the coproporphyrin III concentration in the urines of 7017 and 7018 excreted during the 18th to the 19th November, 1937, when the examination was made it amounts to 0·111 mg. per 100 c.c. and 0·092 mg. per 100 c.c respectively. This is much higher than the daily average total coproporphyrin concentration of 0·003 mg. per 100 c.c. in the urine of the normal animal 6384 (see Table 6). This suggests that the affected animals excrete increased amounts of both copro I and copro III, but unfortunately we have not been able to establish this definitely as up to now it was not possible to determine the presence of these pigments in faeces and urine of a sufficient number of normal bovines when the same animals are examined at different periods during the year.

Summary.

1. In addition to the daily fluctuation in the amount of porphyrin excreted, porphyrin was found to be excreted at a higher level in two affected animals during the late summer than during the early spring. This was found to be due to a greater porphyrin concentration in the faeces and urine than to an increased amount of faeces or urine.

2. When quantitative porphyrin determinations were made over a period of 10-14 days and the daily averages calculated, it was found that the ratio of uro- plus coproporphyrin to coproporphyrin to uroporphyrin was approximately constant except in one case, where during one period abnormally large amounts of urine were excreted.

3. The ratio of coproporphyrin I to coproporphyrin III varies from animal to animal in both the faeces and the urine of two affected animals (7017 and 7018), one suspicious case of the disease (7597) and one apparently normal animal (7022) which is however related to congenital porphyrinuria cases.

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