The Effect of Environmental Temperature on the Urinary Excretion of Riboflavin by the Dog

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In a recent investigation in these laboratories dogs of various breeds and body-weight, and of either sex, were maintained in metabolism cages on a standard diet and standard water intake and their urinary excretion of thiamine was measured (Worden, Waterhouse & Partington, 1954). It was found that, when under these conditions, the environmental temperature was increased by approximately 30° F, the volume of urine decreased to approximately one-third, and that, although the concentration of thiamine in this reduced urine volume was greater, there was a significant drop in its total daily urinary excretion.

During previous investigations with dogs in metabolism cages under standard conditions, it had been noted on a number of occasions that the urinary excretion of riboflavin varied with environmental temperature. During experiments in which environmental temperature was not controlled, whenever the atmospheric temperature became warmer there was a corresponding daily increase in urinary riboflavin output, suggesting a lower requirement at the higher temperatures. It was therefore decided to investigate this temperature effect further.

EXPERIMENTAL

The observations were made on three dogs (two male, one female) accommodated in metabolism cages; samples were collected as previously described. The identification numbers of the dogs employed in the previous studies have been retained (Worden *et al.* 1952, 1954).

The riboflavin contents of the urine were determined in volumes containing approximately 15 μg; the samples were acidified with 0·2 ml. hydrochloric acid (36% (w/w) HCl). The mixture was extracted with chloroform to remove extraneous soluble material, and the aqueous layer treated by the procedure described by Kodicek & Wang (1949).

During the experiment each dog received a measured quantity of a diet composed of pressure-cooked rabbit meat, wholemeal bread and milk. The amounts fed are given in Table 1: the riboflavin content was determined each day by the method of Kodicek & Wang (1949) in a sample from the homogenized mixed diet. Urinary inorganic phosphate was estimated by the method of King (1932).

For the first five consecutive 24h periods the dogs were maintained within a lower environmental temperature range. At the end of the fifth 24h period the environmental temperature was raised, and all observations on the five subsequent 24h periods were assigned to the higher environmental temperature range.

Details of the individual dogs and of their daily dietary allowances are summarized in Table 1.

Table 1. *Details of individual dogs and their daily dietary allowances*

Dog no. and name	Breed	Sex	Age (years)	Weight at beginning of test (kg)	Weight at end of test (kg)	Dietary allowance/24h: Cooked rabbit meat (g)	Dietary allowance/24h: Wholemeal bread (g)	Dietary allowance/24h: Milk (ml.)	Dietary allowance/24h: Water (ml.)
4, Scottie	Scottish terrier	Male	3	8·6	9·5	227	170	250	500
1, Pat	Mongrel terrier, smooth-coated	Male	4	22·2	22·6	227	170	250	750
7, Roxy	Cocker spaniel, black and white	Female	6	14·5	12·2	170	127·5	187·5	500

RESULTS

The general condition and behaviour of dog no. 4 (Scottie) during the experiment were excellent, even during the latter part, when the environmental temperature was considerably raised. He was bright and active in the cage—in which he had room to run and jump about—and in this respect his behaviour differed markedly from that of dog no. 1 (Pat), a more placid animal, although apparently quite happy throughout the test. Although Pat was more than twice the weight of Scottie he required only the same weight daily of the standard diet to satisfy him. Dog no. 7 (Roxy) could not be persuaded to consume voluntarily as much food as the other two dogs, since she has normally a smaller appetite, and her food intake throughout the experiment was therefore adjusted to 75% of that of dogs nos. 1 and 4. The concentration of riboflavin in her urine expressed as μg/ml. was somewhat variable; also, owing to the variations in volume of urine voided per 24h, her daily excretion of riboflavin at the lower environmental temperatures fluctuated considerably.

In all three animals the urinary volume excreted per 24h at the higher environmental temperatures fell to between one-third and one-half of that at the lower, the remaining water being lost presumably by increased evaporation from the body surfaces.

The values obtained when each of the three dogs was confined to a metabolism cage for a 10-day period are given in Table 2.

In Table 3 the average daily riboflavin output at low and high environmental temperatures is compared for each dog under study; it will be seen that the differences for dogs no. 4 (Scottie) and no. 1 (Pat) are significant at $P<0{\cdot}01$, but for dog no. 7 (Roxy) they are not significant ($0{\cdot}4>P>0{\cdot}3$).

The results obtained with dog no. 7 emphasize the important effect of irregular urinary volumes in experiments of this type. The daily fluctuations in volume are attributed to psychological factors leading to periodical retention; when an animal

Table 2. *Dietary intake and urinary excretion of riboflavin and other results for dogs in metabolism cages at different environmental temperatures*

Dog no. and name	Date	Riboflavin intake		Values for urinary samples collected at end of each 24h in metabolism cage						Temperature within metabolism cage (24h readings)	
				Volume of sample	Specific gravity	Riboflavin		Inorganic phosphate (as P)		Minimum thermometer	Maximum thermometer
		μg/g diet	μg/24h	(ml.)		μg/ml.	μg/24h	mg/ml.	mg/24h	(° F)	(° F)
4, Scottie	8. vi. 53	1·20	960	—	—	—	—	—	—	—	—
	9. vi. 53	1·39	1108	730	1·000	0·28	204	0·38	278	57	64
	10. vi. 53	1·15	915	780	1·000	0·29	226	0·53	415	61	68
	11. vi. 53	1·15	915	520	1·005	0·40	208	0·66	344	60	60
	12. vi. 53	1·00	796	668	0·999	0·27	180	0·79	525	60	62
	13. vi. 53	1·30	1040	596	1·000	0·44	262	0·82	490	70	76
	14. vi. 53	1·40	1115	532	1·023	0·43	230	0·77	409	77	78
	15. vi. 53	0·90	717	360	1·031	1·17	420	1·25	448	79	102
	16. vi. 53	1·35	1075	382	1·033	1·00	382	1·26	480	80	92
	17. vi. 53	1·35	1075	232	1·036	1·82	422	1·41	327	84	92
	18. vi. 53	—	—	272	1·044	1·46	397	1·68	456	76	94
1, Pat	9. ix. 53	1·09	867	—	—	—	—	—	—	—	—
	10. ix. 53	1·40	1110	680*	1·022	0·40	262	0·45	306	64	74
	11. ix. 53	1·00	786	1060	1·022	0·26	276	0·45	477	54	62
	12. ix. 53	1·25	983	850	1·020	0·28	238	0·63	530	62	62
	13. ix. 53	1·10	865	990	1·020	0·24	238	0·68	668	54	68
	14. ix. 53	1·10	865	824	1·017	0·22	181	0·28	233	54	68
	15. ix. 53	1·40	1110	517	1·018	0·58	300	0·86	445	78	82
	16. ix. 53	1·04	820	680	1·022	0·48	326	0·76	514	83	84
	17. ix. 53	†	†	520	1·023	0·66	343	0·98	517	81	85
	18. ix. 53	1·21	950	826	1·019	0·44	364	0·69	576	78	86
	19. ix. 53	—	—	600	1·028	0·75	450	0·84	501	78	86
7, Roxy	16. vii. 53	1·14	682	—	—	—	—	—	—	—	—
	17. vii. 53	1·47	880	656	1·025	0·40	262	0·86	560	68	70
	18. vii. 53	1·31	785	668	1·008	0·34	227	0·62	413	68	70
	19. vii. 53	1·00	598	666	1·022	0·70	465	1·28	852	60	72
	20. vii. 53	1·25	750	254	1·018	1·30	330	0·63	159	68	72
	21. vii. 53	1·00	598	608	1·020	0·78	474	0·92	556	68	70
	22. vii. 53	1·34	802	116	1·035	1·25	145	1·54	179	80	87
	23. vii. 53	1·04	622	250	1·034	1·66	415	1·59	398	86	88
	24. vii. 53	0·91	545	280	1·035	2·19	614	1·67	467	84	90
	25. vii. 53	0·95	568	256	1·035	2·10	536	1·72	440	88	90
	26. vii. 53	—	—	170	1·049	3·52	598	2·46	418	88	90

The breaks within the columns indicate change from lower to higher environmental temperature.
* Reduced volume—dog escaped from metabolism cage. † Faulty reading.

tends to be 'nervous' and sensitive the effect is considerable. Such findings as those on dog no. 7 emphasize the need to relate output to periods much longer than 24h for consistent and repeatable results (see Worden, 1939; Worden *et al.* 1952). Over the last 4 days at the higher temperature, by which time the daily urinary volume fluctuated much less, the standard deviation of daily riboflavin excretion by dog no. 7 was nearer to those of dogs nos. 1 and 4.

Table 3. *Mean values with their standard errors for 24h urinary riboflavin excretion of three dogs housed in metabolism cages at different environmental temperatures*

	At lower environmental temperatures (first five consecutive 24h periods)			At higher environmental temperatures (second five consecutive 24h periods)			Significance of difference between means for lower and higher environmental temperatures	
Dog no. and name	Range of minimum thermometer readings (° F)	Range of maximum thermometer readings (° F)	Mean urinary riboflavin output (μg/24h)	Range of minimum thermometer readings (° F)	Range of maximum thermometer readings (° F)	Mean urinary riboflavin output (μg/24h)	t	Significance
4, Scottie	57–70	60–76	216·1 ± 13·57	76–84	78–102	370·2 ± 35·83	4·013	$P < 0·01$
1, Pat	54–64	62–74	239·0 ± 16·22	78–83	82–86	356·6 ± 25·54	3·875	$P < 0·01$
7, Roxy	60–68	70–72	351·6 ± 50·92	80–88	87–90	461·6 ± 86·54	1·09	$0·4 > P > 0·3$

The urinary levels of inorganic phosphate were determined primarily in order to compare fluctuations in riboflavin excretion with those of constituents that have received wider study. The daily excretion of inorganic phosphate was parallel with that of riboflavin. This apparent relationship has been noted in a whole series of metabolism trials; it is hoped to discuss it in a later publication.

DISCUSSION

Beher & Gaebler (1950) studied the daily urinary excretion of riboflavin in adult bitches of various body-weights and adapted to periods of confinement in metabolism cages. They found that the excretion of riboflavin was different for animals receiving the same amounts of the same diet, but that in a given dog the administration of testosterone propionate lowered the amount of riboflavin excreted, owing presumably to the anabolic effects of the male hormone in the female animal. Even more pronounced results were recorded for the urinary excretion of *N'*-methylnicotinamide, and there was a similar but less marked trend in the urinary excretion of ascorbic acid.

The degree of utilization of riboflavin by the tissues must clearly be influenced by the metabolic rate, and this will consequently affect the proportion of any given intake that will be excreted in the urine. In a given animal, however, it is reasonable to assume on the basis of current knowledge about the physiological functions of riboflavin that the amount required will depend principally on the nutrients utilized. With the increased calorific demands of a colder environment it would be expected that the utilization of riboflavin also would be increased.

The work of Feder, Lewis & Alden (1944) suggests a relationship between urine volume and riboflavin output through the kidneys. In our experiments, however, there was a large decrease in daily output of urine at the higher environmental

temperature, and it coincided with an increase in daily riboflavin output. It seems evident, therefore, that in a warm environment the loss of water from the body by routes other than the kidney has no effect on the excretion of riboflavin.

Other workers have attempted to determine the effect of temperature on riboflavin requirements. Mills (1943) used growing rats on different levels of riboflavin in a cool room at 65° F and in a hot room at 91° F. Since the minimum level of riboflavin inducing the most rapid average gain in groups of four rats seemed the same in both environments (2 mg/kg diet), he concluded that there was no effect on riboflavin requirements. It is difficult to assess the results, as the animals were fed *ad lib.*, but on the basis of average food economy it would seem that the riboflavin requirement in the hot room was near 1 mg/kg diet.

Mitchell, Johnson, Hamilton & Haines (1950) obtained results with young pigs that clearly showed riboflavin requirements inversely related to the environmental temperatures. They found the requirements in the total ration to be 1·2 p.p.m. or less at 85° F and approximately 2·3 p.p.m. at 42° F. These values were equivalent to 0·54 and 1·04 mg riboflavin/lb. feed respectively. The authors confirmed this effect of temperature with preliminary experiments on older pigs, when on the same diet they found consistently less riboflavin in the urine at 42° F than at 85° F.

In view of these findings with the rat or the pig as test animal, and of the results now obtained on the dog, it would appear to be important to carry out work involving quantitative assessment of riboflavin requirements under reasonably standard conditions, or at least that the conditions of the experiment should be clearly stated. In the field of human nutrition many attempts have been made to evolve a quantitative method for the diagnosis of riboflavin deficiency. Feder *et al.* (1944) considered that an excretion of less than 0·3 μg riboflavin/ml. urine was indicative of riboflavin deficiency, and the riboflavin concentration in a sample of fasting morning urine was claimed to be as valuable as a saturation test for estimating the degree of riboflavin deficiency. Other workers have used a saturation test. For instance, Oldham, Johnston, Kleiger & Hedderich-Arismendi (1944) reported a close relationship between the excretion rate and the response to a test dose. They suggested that the elimination of 1 μg/h by a fasting subject, or the elimination of at least 20% of a test dose within 4 h of its administration, showed an adequate nutritional status. On the other hand, Axelrod, Spies, Elvehjem & Axelrod (1941) claimed that the test-dose method was unsatisfactory, and obtained no correlation between the percentage of test dose retained and the amount excreted in the urine. It would be of interest to know whether environmental temperature plays a role in the riboflavin requirement of the human subject. If it does, then on similar diets the riboflavin excretion during winter would be much lower for a given subject than at higher temperatures during summer. It would therefore seem essential to lay down detailed conditions of test in any attempt at a quantitative assessment of riboflavin deficiency. Montenero & Frongia (1951) have already reported a considerable rise in riboflavin excretion in five healthy subjects after the artificial induction of a temperature of 39° for 3 days, the riboflavin excretion subsequently returning to normal.

SUMMARY

1. The urinary excretion of riboflavin was followed in three adult dogs of different body-weights, housed in metabolism cages and receiving known amounts of riboflavin in a standard diet.

2. In each instance the excretion of riboflavin could be altered by varying the environmental temperature. With an increase of approximately 20° F in the environmental temperature the urine excreted was reduced to between one-third and one half of the original volume. The concentration of riboflavin in the reduced volume of urine excreted at the higher environmental temperature was, however, always more than twice that in the urine excreted at the lower environmental temperature. The total daily urinary excretion of riboflavin in two of the dogs was about 75%, and that in the third dog about 25%, greater at the higher environmental temperature.

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