cretion is accounted for entirely by urea nitrogen, for there is no change in the excretion of ammonia nitrogen, creatinine, creatine, or uric acid. Fecal nitrogen excretion falls with the decreased intake in the vitamin B_1 deficient animals as well as in the paired feeding controls.

Total urinary sulfur excretion shows nearly the same absolute value during the deficiency as during the control period, but expressed in percent of intake it parallels the excretion of nitrogen, rising from 21 to 48%. There is hardly any change in the total sulfates as expressed in percent of the total urinary sulfur excretion. The rise in neutral and ethereal sulfur, indicating a disturbance in endogenous sulfur metabolism, is the same as that observed in the paired feeding controls. Fecal sulfur excretion drops with the decreased intake, but expressed in percent of intake it remains unchanged.

It is apparent that the only significant disturbance in metabolism in vitamin B_1 deficient animals is in the increased urinary nitrogen and sulfur excretion which exceeds, to a considerable extent, the increased excretion of nitrogen and sulfur observed in the paired feeding controls.

Though vitamin B_1 is significant in oxidative processes in cells of the central nervous system and possibly in cellular metabolism in general, its complete depletion is not associated with disturbances in the metabolism of most of the essential elements of the diet.

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Presence of an Antagonistic Factor in Serum of Dogs Following Repeated Injections of Cortin.*

C. GWENDOLINE TOBY AND LENA A. LEWIS. (Introduced by F. A. Hartman.)

From the Department of Physiology, The Ohio State University, Columbus.

It has been reported[1] that in the normal dog a marked decrease in the excretion of Na and usually an increase in the excretion of K occurs during a 6-hour period following the intravenous injection of 20-40 cat units of cortin; and that with repeated injections (at

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[1] Hartman, F. A., Lewis, L. A., and Toby, G., *Science*, 1937, **86**, 128.

intervals of several days), the effect becomes progressively less, until there is little change in electrolyte excretion following administration of the extract. It has since been found that NH_3 excretion is also increased following cortin and that with repeated injections this response is decreased. Of 9 animals injected with 20-40 cat units of cortin, all gave the characteristic electrolyte response described (Table I). Four different cortical extracts prepared in this laboratory were used. The response elicited by a given extract in different dogs was very similar (Table I). Five of these animals were injected until they became completely refractory.

TABLE I.
Initial Response to Intravenously Injected Cortin in the Normal Female Dog.

Dog	Treatment	m. eq. excreted in 6 hr. Na		K	NH_3-N	
I	Controls (6)	8.71	(6.18-10.18)*	5.11	6.35	(2)
	Cortin 1	1.24		6.25	11.00	
III	Controls (4)	10.51	(7.64-14.01)	8.31	5.75	(2)
	Cortin 1	1.15		8.62	12.50	
IV†	Controls (3)	9.76	(8.25-11.21)	8.64	5.20	(2)
	Cortin 1	3.21		8.36	9.40	
V	Controls (2)	12.50	(11.30-13.69)	5.56	6.35	(1)
	Cortin 2	6.65		6.05	7.93	
VIII	Controls (2)	15.55	(13.67-17.43)	6.20	6.35	
	Cortin 2	6.98		6.43	12.11	
XIII	Controls (4)	17.33	(15.00-19.44)	9.12	7.28	(2)
	Cortin 2	11.86		9.84	10.65	
XIX	Controls(4)	12.23	(10.24-13.80)	7.48	7.14	
	Cortin 3	6.80		8.60	13.00	
VII	Controls (3)	15.71	(13.82-19.29)	6.79	5.53	
	Cortin 4	7.98		7.81	7.43	
Average change after cortin		−7.05		+0.60	+4.26	

*range.
†one previous injection.

Failure of these animals to respond to further injections suggested the possibility that some antagonistic substance was present in the blood. Using sterile precautions, blood was taken from 2 dogs (I and III), which had received 7 and 11 injections respectively over periods of 5 and 9 weeks. The serum was separated and stored at 4°C. Five untreated animals were given 10-25 cc. of this serum intravenously about 15 minutes previous to the injection of the usual amount of cortin. In every case in which cortin and serum were given, the Na and the NH_3 did not change beyond the range of the controls, whereas cortin alone always reduced the Na to a level well below that of the lowest control and increased the NH_3 well above the highest control value (Table II). Decreases in Na of 86-89% in 3 animals (I, III, X) were shown when 0.5 cc. (20

C.U.) of extract 1 was given. The same amount of this extract given with serum from refractory dogs decreased the Na excretion by only 14 and 17% (XI, XII). Extract 2 (40 C.U.) gave decreases of 32-55% in 3 animals (V, VIII, XIII). When injected following serum, the same amount of this extract gave, in one instance, an increase of 5% over the control levels (XIV) and in another, a decrease of 1% (XV). In one case where only 10 cc. of the serum was used with extract 2, there was a decrease of 17% in Na excretion (XVI). The injection of serum from normal dogs had no inhibitory effect (IX and X). Since the range for K and NH_3 was small and comparable with the range of Na these values are not included in the table.

TABLE II.
Initial Response to Intravenously Injected Serum and Cortin in the Normal Female Dog.

Dog	Treatment	m. eq. excreted in 6 hr. Na		K	NH_3-N
X	Controls (4)	11.17	(7.14-16.37)*	6.60	6.50
	Last control before inj.	10.60		5.95	7.55
	Normal serum—18 cc.				
	Cortin 1	2.15		6.70	10.10
IX	Controls (2)	6.30	(5.90-6.70)	5.21	5.58
	Last control before inj.	5.90		5.09	5.58
	Normal serum—20 cc.				
	Cortin 3	3.04		5.85	7.45
XI	Controls (2)	9.61	(6.04-13.19)	5.57	5.92
	Last control before inj.	6.04		6.04	6.00
	Sera I and III—23 cc.				
	Cortin 1	7.98		5.52	6.56
XII	Controls (3)	16.35	(11.96-20.70)	9.26	5.50
	Sera I and III—18 cc.	13.98		8.68	5.57
	Cortin 1				
	Control after inj.	11.96		10.16	6.50
XIV	Controls (3)	11.87	(9.46-14.08)	8.12	9.80
	Last control before inj.	12.08		8.41	9.06
	Sera I and III—25 cc.				
	Cortin 2	17.90		9.20	7.35
XV	Controls (4)	11.56	(11.06-11.90)	6.73	6.35
	Last control before inj.	11.06		6.17	6.42
	Serum I—25 cc.				
	Cortin 2	11.43		6.98	6.35
XVI	Controls (8)	9.38	(7.34-11.67)	6.59	7.50
	Last control before inj.	7.92		6.30	8.15
	Sera I and III—10 cc.				
	Cortin 2	7.80		7.00	7.70
Aver. change after serum and cortin		+0.34		+0.22	—0.35

*range.

Serum obtained from dogs I and III several weeks after the last injection of cortin still demonstrated this inhibitory effect. Storage at 4°C. for 2-3 weeks did not destroy the antagonistic factor.

The methods for analyses of Na and K were those previously described.[2] NH_3 was determined by Sobel, Yuska and Cohen's modification of Van Slyke and Cullen's method.

The cortical extracts used were highly purified, contained very little solid, were free from protein and contained practically no nitrogen. Potent extracts gave a precipitin reaction with serum from refractory dogs, but did not react with normal dog serum. Preliminary experiments, using cortin as the antigen, would indicate that the serum of repeatedly injected animals may have some power of fixing complement.

We wish to express our thanks to Dr. F. A. Hartman for his helpful criticism and advice throughout the course of these experiments.

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Determination of Amino-Nitrogen in Urine.

F. A. TAYLOR. (Introduced by S. R. Haythorn.)

From the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.

Despite the importance of an understanding of amino-acid metabolism, there has been no trustworthy method of amino-nitrogen determination that could be easily applied as a routine in a clinical laboratory. The chief difficulty has been in the preparation of filtrates of blood and urine for the actual titrations. In this note a method is described for the removal of NH_3 and CO_2 from urine filtrates enabling one to determine amino-nitrogen expeditiously by the Sörensen titration. Many determinations may be carried out in parallel with little supervision by the analyst.

The Sörensen[1] titration as described by Northrop[2] is used. The preparation of urine filtrates follows the procedure of Van Slyke and Kirk.[3] Instead of distilling the NH_3 and CO_2 successively *in vacuo*, the filtrates are exposed in shallow layers *in vacuo* in a desiccator over dilute H_2SO_4. This means of collecting or removing NH_3 has been known for a long time and occasional reference is

2 Thorn, G. W., Garbutt, Helen R., Hitchcock, F. A., and Hartman, F. A., *Endocrin.*, 1937, **21**, 213.

1 Sörensen, S. P. L., *Biochem. Z.*, 1908, **7**, 45.

2 Northrop, J. H., *J. Gen. Physiol.*, 1926, **9**, 767.

3 Van Slyke, D. D., and Kirk, E., *J. Biol. Chem.*, 1933, **102**, 651.