The ether solution was evaporated and a red oil of a peculiar odor was obtained. It was fractionated *in vacuo*. The larger fraction boiled at 144° and 26 mm. A number of analyses indicated that the oil is a mixture, approximating the percentages of composition of $C_6H_5C \equiv CI$; however, since all the products cannot be accounted for by an equation, such as:

$$2C_6H_5NHNH_2 + C_2I_2 \longrightarrow C_6H_5NHNH_2HI + C_6H_5C \equiv CI + N_2 + H_2,$$

other experiments must be made.

Other bases than the above-mentioned react with diiodoacetylene in ether solution and give well characterized crystallin products. These will be discussed in a future contribution.

SEATTLE, WASHINGTON.

[FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS.]

FASTING STUDIES: IV (STUDIES ON WATER DRINKING: VII). ON THE ALLANTOIN AND PURINE EXCRETION OF FASTING DOGS.

BY S. R. WREATH AND P. B. HAWK.

Received July 25, 1911.

Introduction.

The occurrence of allantoin as a urinary constituent was first demonstrated by Wöhler.[1] He found it to be present in the urine of suckling calves in sufficient quantity to separate out in crystallin form. The role played by allantoin in the metabolic processes of the animal organism, however, did not receive serious attention until about 50 years after Wöhler had made his pioneer observation. In fact for years it was believed that allantoin was confined, as an excretory product, to the urine of infants and the young of certain species of lower animals, among them the calf, as already mentioned.

It was early known, of course, that allantoin could be produced *in vitro* by the oxidation of uric acid. With this relation in mind, Wöhler and Frerichs[2] fed uric acid to dogs and rabbits as well as to men and searched for allantoin in the urine of the subjects. They were unsuccessful in their attempts to isolate the substance and consequently came to the conclusion that the uric acid which they had fed had undergone a profound oxidation in the animal body and that the final oxidation products were unknown substances below the allantoin stage. Several years later Neubauer[3] likewise made an unsuccessful attempt to demonstrate the presence of allantoin in the urine of rabbits after uric acid

[1] Wöhler, *Ann.*, **70**, 229 (1849).

[2] *Ann.*, **65**, 335 (1848).

[3] *Ibid.*, **99**, 217 (1856).

feeding. Finally, in 1876, Salkowski[1] isolated fairly large quantities of allantoin from the urine of dogs which had been fed uric acid, and Minkowski[2] later confirmed this observation.

Our knowledge regarding the metabolic cycle of which allantoin is the end product has been greatly advanced through the investigations of Mendel[3] and his associates coupled with the work of Cohn,[4] Salkowski,[5] and Swain.[6] These investigators have demonstrated beyond question that an increased output of allantoin follows the introduction not only of uric acid but of purine derivatives such as free purine bases or the nucleic acids or their salts into the organism of the cat or dog. More recently Wiechowski[7] has secured allantoin as an oxidation product of uric acid through the enzymic power of extracts of the liver of the dog and the kidney of the ox. The yield of allantoin was nearly quantitative in these tests. Furthermore, Minkowski[8] and Paduschka[9] have shown that allantoin ingested by dogs may be obtained unchanged and practically quantitatively in the urine. This finding is further emphasized through the recent report of Schittenhelm and Wiener,[10] to the effect that 30 per cent. of the total quantity of allantoin ingested by human subjects may be obtained in the urine. These experiments just mentioned would seem to indicate clearly that we may with justice consider allantoin as an end-product of the oxidation of purine substances, at least in the human organism and in that of the dog. The finding of allantoin in the urine of fasting dogs by Underhill and Kleiner[11] and by Schittenhelm[12] and Wiechowski[13] strengthened the claim of allantoin to consideration as an end-product of purine metabolism. The uncertainty which formerly existed as to the normal occurrence of allantoin has been entirely dissipated through the interesting series of experiments by Wiechowski,[14] who has demonstrated the presence of allantoin in the

[1] *Ber.*, **9**, 719 (1876).

[2] *Arch. exp. Path. Pharm.*, 41, 398 (1898).

[3] Mendel and Brown, *Am. J. Physiol.*, **3**, 261 (1900). Mendel and Jackson, *Ibid*, 4, 163 (1900). Mendel, Underhill and White, *Ibid.*, 8, 377 (1902–3). Mendel and White, *Ibid.*, **12**, 85 (1904–5).

[4] *Z. physiol. Chem.*, **25**, 507 (1898).

[5] *Centr. Med. Wiss.*, 929 (1898).

[6] *Am. J. Physiol.*, **6**, 38 (1901).

[7] Hofmeister's *Beitr.*, 4, 295 (1907)

[8] *Arch. exp. Path.*, 41, 375 (1898).

[9] *Ibid.*, 44, 19 (1900).

[10] *Z. physiol. Chem.*, **63**, 283 (1909).

[11] *J. Biol. Chem.*, **4**, 165 (1908).

[12] *Z. physiol. Chem.*, **62**, 80 (1909).

[13] Hofmeister's *Beitr.*, 11, 109 (1908).

[14] Hofmeister's *Beitr.*, **11**, 109 (1908); *Arch. exp. Path. Pharm.*, **60**, 185 (1909); *Biochem. Z.*, **19**, 368 (1909.)

urine of the dog, rabbit, cat, monkey, horse and man. This author has likewise elaborated a method for the determination of allantoin which is probably far more accurate than any method previously in vogue for this purpose.

Salkowski[1] has found allantoin to be present in the urine of the cow, whereas Swain[2] has isolated it from coyote urine, the latter observation having been very recently verified by Hunter and Givens.[3] The finding of allantoin in monkey urine has been verified by Wells.[4] According to Wiechowski allantoin is the only oxidation product of uric acid which appears in the urine following the subcutaneous injection of uric acid. In the case of rabbits, from 56.4 per cent. to 92 per cent. of the allantoin equivalent of the uric acid injected was recovered in the urine.

Certain experiments of Abderhalden, London and Schittenhelm[5] are of interest in connection with nuclein metabolism. They worked upon dogs after the establishment of the Eck fistula.[6] They found that the elimination of the liver function by this means had no influence upon the decomposition of nucleic acid and the subsequent deamidation and oxidation of the purine bases. There was, however, a disturbance in the transformation of uric acid into allantoin, thus indicating a diminution in the uricolytic function of the liver. The following distribution of purine nitrogen was noted in Eck fistula dogs as compared with normal animals:

	Allantoin. Per cent.	Purine bases. Per cent.	Uric acid. Per cent.
Normal	94–97	1–2	2–4
Eck fistula	74–87	1–2.5	12–25

It is now generally believed that allantoin is the principal end-product of purine metabolism in practically all lower animals. This relation has not been established for the human organism, however. Even in the case of man, however, the metabolic relations of allantoin are assuming a new and increasing importance. The researches of Wiechowski, already referred to, aided by his excellent method for the accurate determination of the urinary constituent, have been the most important factors in demonstrating the actual role of allantoin in metabolism.

The allantoin present in the urine originates from two sources. Abundant evidence of *exogenous* allantoin has been furnished in the many feeding experiments to which reference has already been made. The ex-

[1] *Z. physiol. Chem.*, **42**, 213 (1904).
[2] *Am. J. Physiol.*, **13**, 30 (1905).
[3] *J. Biol. Chem.*, 8, 449 (1910).
[4] *Ibid.*, **7**, 171 (1910).
[5] *Z. physiol. Chem.*, **61**, 413 (1909).
[6] Eck, *Militär-Medicinischer J.*, **132**, 1877. Hahn, Massen, Nencki and Pavlov, *Arch. exp. Path. und Pharm.*, **32**, 161 (1893). Sweet, *J. Exp. Med.*, **6**, 161 (1905). Hawk, *Am. J. Physiol.*, **21**, 259 (1908).

perimental bases for the claims of an endogenous allantoin excretion are more meagre but none the less convincing. The first experimental evidence so far as we are aware which indicated an endogenous source of allantoin was the report of Underhill and Kleiner,[1] to the effect that they had found that substance in the urine of two fasting dogs. They could, however, demonstrate no uniformity in the daily output of allantoin nor any relation between the allantoin excretion and the purine output. About the same time Wiechowski[2] made an examination of the urine of dogs after short fasts and reported a fairly uniform allantoin output from day to day. Later came the report by Schittenhelm[1] of a similar finding. The percentage of the total nitrogen which was excreted in the form of allantoin, however, decreased during the final days of the fast. The last work upon the allantoin excretion of the fasting organism is that very recently reported by Hunter and Givens[1] in which they show the daily output of a fasting coyote to be very uniform from day to day of an eight-day fast.

Only a portion of the purines of the diet appear in the urine as uric acid. A portion is further oxidized and appears in the form of allantoin. The action of nucleases, deamidizing and oxidizing enzymes, upon the nucleic acids and purine compounds are factors in influencing the output of purine nitrogen in the urine. The problem of uric acid formation, decomposition and retention also has an important bearing. So far as the urinary purine base excretion is concerned, the most extensive investigations have been made by Krüger and Salomon.[3] They isolated the following quantities of purine bases from 10,000 liters of human urine: 1-Methyl-xanthine, 31.32 g.; heteroxanthine, 22.35 g.; paraxanthine, 15.32 g.; xanthine, 10.12 g.; hypoxanthine, 8.51 g.; adenine, 3.54 g.; epiguanine, 3.4 g. The feeding of meat, thymus, spleen, etc., produces an increased output of purine bases in the urine.

The purine excretion of fasting animals has been observed in but few instances. The investigations of Underhill and Kleiner, Schittenhelm and Hunter and Givens[4] are the most important. The first two investigations were made upon dogs, whereas a coyote was the subject of the third experiment. Underhill and Kleiner showed the output of purine nitrogen to be irregular during a thirteen-day fast with no decided tendency toward either a decrease or an increase as the fast progressed. The same in general is true of Schittenhelm's findings. In the work of Hunter and Givens no attempt was made to follow the course of the purine output, the analyses being made on a composit urine sample.

[1] *Loc. cit.*

[2] Hofmeister's *Beitr.*, 11, 109 (1908).

[3] *Z. physiol. Chem.*, **24**, 364 (1897); *Ibid.*, **26**, 350 (1898).

[4] Underhill and Kleiner, *Loc. cit.* Schittenhelm, *Loc. cit.* Hunter and Givens, *Loc. cit.*

A very interesting and important relationship has been observed by Schittenhelm and by Hunter and Givens between the allantoin and purine excretions. The first mentioned investigator, for example, after feeding 0.26 gram of purine nitrogen to a fasting dog, recovered 0.25 gram in the urine of the following 24 hours. This was 96 per cent. of the ingested amount. It is very significant that of this 96 per cent., allantoin furnished 95 per cent., purine bases 4 per cent., and uric acid 1 per cent. In the case of the fasting coyote Hunter and Givens found 95.7 per cent. of the nitrogen of purine origin to be excreted in the form of allantoin, whereas but 4.3 per cent. was eliminated as urinary purine nitrogen; when the coyote was fed a meat diet the corresponding percentages were 97.2 and 2.8, respectively.

Plan and Methods.

In the present paper data are given for the allantoin and purine excretions of three fasting dogs and a comparison made between these data and those already reported[1] from a fourth fasting dog. Two of the three dogs under investigation (dogs 2 and 3) were adult males, whereas the third (dog 6) was a male pup one month old. The fasts of the adult dogs were 48 and 96 days in length, respectively, whereas the pup fasted only a week. Previous to the fast the adult animals were fed a diet of meat, cracker meal, lard and bone ash; the pup was given a milk ration. The quantities of the various dietary constituents fed these animals during each day of the preliminary period are listed in Table I. For

TABLE I.—DAILY DIETS.

(Grams.)

	Meat.	Cracker dust.	Lard.	Bone ash.	Water.
Dog No. 2	250	70	30	10	500[2]
Dog No. 3	400	100	45	12	700[2]
Dog No. 6	100 cc. of milk[3]				

chemical analysis of the foods see the paper by Howe and Hawk,[4] already mentioned. The body weights (kg.) of the animals were as follows:

	End of preliminary period.	End of fast.
Dog No. 3	26.33	11.32
Dog No. 2	13.64	6.42
Dog No. 6	1.34	1.05

In each instance approximate nitrogen equilibrium was secured during a preliminary period in which the dogs were caused to ingest a uniform

[1] Howe and Hawk, THIS JOURNAL, **33**, 215 (1911).

[2] During each day of the fast the volume of water was fed by means of a stomach tube.

[3] During the fast this animal received 100 cc. water per day by means of a stomach tube.

[4] THIS JOURNAL, **33**, 215 (1911).

diet of constant water content.[1] When a satisfactory nitrogen balance was secured the fast began.

So far as possible, each individual 24-hour urine sample was subjected to analysis. In a few instances, and particularly in the case of dog No. 3, composit samples of urine were analyzed, including aliquot portions from the urinary output of periods ranging from 5 to 10 days in length. The method used for the determination of allantoin and purine nitrogen was that of Paduschka[2] as modified by Underhill and Kleiner.[2] Unfortunately, the superior method of Wiechowski was not reported until we were well along on our experiment. In another investigation now under way in this laboratory Wiechowski's method is being employed.

In Tables II, III and IV will be found the data for the allantoin and purine excretions of dogs Nos. 2, 3, and 6, whereas Table V contains data from the fourth dog already mentioned.

Discussion of Results.

Allantoin Excretion. Allantoin Excretion of Dog No. 3.—The data concerning the allantoin output of this animal will be found in Table II. An examination of this table will show that the average daily output of allantoin nitrogen for the feeding period was 0.051 gram, or 0.22 per cent. of the total nitrogen output for that time. The elimination of nitrogen in the form of allantoin during the course of the 96-day fast was irregular. In general, however, there was a tendency toward a *decreased allantoin excretion* as the fast progressed. This is shown very clearly; for example, if we compare the total output of allantoin-nitrogen for thirty days in the early part of the fast with the output for a similar period at the end of the fast. The actual output of allantoin-nitrogen for thirty days early in the fast was 0.4 gram, whereas the similar value for the latter part of the 96-day fast was 0.24 gram. It is evident, therefore, that this animal excreted only 60 per cent. as much nitrogen per day in the form of allantoin during the advanced stages of fasting as was eliminated during the early stages of the period. As compared with the feeding period, the values for the average daily allantoin-nitrogen excretion are as follows: Feeding period = 0.051 gram; early stage of fasting (4–38 days) = 0.013 gram; late stage of fasting (67–96 days) = 0.008 gram. None of the other investigators mentioned as having investigated the allantoin excretion of fasting animals[3] have reported a pronounced decrease of this nature in the allantoin excretion in the final stages of the fast. This

[1] Fowler and Hawk, *J. Exp. Med.*, 12, 388 (1910). Hawk *et al.*, Proceedings of the Second International Congress of Alimentary Hygiene and the Rational Feeding of Man (Bruxelles, 1910), Vol. I, Section 2, p. 30.

[2] *Loc. cit.*

[3] Schittenhelm, *Loc. cit.* Underhill and Kleiner, *Loc. cit.* Hunter and Givens, *Loc. cit.*

may be due to the fact that the 96-day fast here considered is much longer than the fasting periods utilized by the investigators in question.

TABLE II.—ALLANTOIN AND PURINE EXCRETION.

Dog. No. 3. Daily averages.

Day of expt.	Allantoin-nitrogen. Total. Gram.	Daily average. Grams.	Per cent. of total nitrogen.	Purine-nitrogen. Total. Gram	Daily average. Grams.	Per cent. of total nitrogen.
		Preliminary Period.				
Av. for period		0.051	0.22		0.067	0.29
		Fasting Period.				
1	...	...	....	0.098	0.098	1.05
2	...	...	....	...	...	....
3	...	...	....	...	...	....
4	0.065	0.065	0.72	0.035	0.035	0.39
5	0.025	0.025	0.53	0.020	0.020	0.42
6	...	...	....	...	...	....
7	0.021	0.021	0.68	0.013	0.013	0.42
8	0.028	0.028	0.53	0.021	0.021	0.40
9	...	...	....	...	...	....
10	0.021	0.021	0.34	0.024	0.024	0.39
11	...	...	....	...	...	....
12	...	...	....	...	...	....
13	0.014	0.014	0.34	0.018	0.018	0.44
14	0.007	0.007	0.42	0.010	0.010	0.60
15	0.100	0.100	0.41	0.006	0.006	0.25
16	0.025	0.025	0.53	0.013	0.013	0.28
17	0.008	0.008	0.23	0.020	0.020	0.58
18	...	...	....	0.023	0.023	0.38
19	0.003	0.003	0.24	0.004	0.004	0.32
20–29	0.019	0.002	0.56	0.139	0.014	0.41
30–38	0.066	0.007	0.24	0.114	0.013	0.42
39–49	0.065	0.006	0.20	0.181	0.016	0.56
50–59	0.111	0.011	0.36	0.153	0.015	0.50
60	0.036	0.036	0.71	0.005	0.005	0.10
61	0.024	0.024	0.65	0.004	0.004	0.11
62	0.036	0.036	1.16	0.002	0.002	0.07
63	0.028	0.028	1.00	0.002	0.002	0.07
64	0.016	0.016	0.71	0.002	0.002	0.09
65	0.018	0.018	0.60	0.012	0.012	0.40
66	0.010	0.010	0.46	0.007	0.007	0.32
67–71	0.030	0.006	0.20	0.131	0.026	0.88
72–76	0.031	0.006	0.18	0.036	0.007	0.21
77–81	0.027	0.005	0.20	0.048	0.010	0.36
82–86	0.040	0.008	0.26	0.037	0.007	0.24
87–91	0.048	0.010	0.37	0.047	0.009	0.36
92–96	0.065	0.013	0.51	0.034	0.007	0.27

When we come to consider the percentage of the total nitrogen excretion of the various fasting days which was eliminated in the form of allantoin we find a decrease during the latter part of the fast, which is fairly

comparable to the decrease in the actual allantoin elimination for that period. It is significant that this decrease is secured notwithstanding the fact that the actual output of allantoin, as well as the percentage excretion, is seen to undergo a gradual increase from the 72nd to the 96th day. Even this progressive increase, however, does not cause the allantoin values for the 96th day to equal those of the 64th day. In comparison with the percentage values of the feeding period those of the fast are seen to be greatly *increased*. The ingestion of 2100 cc. of water per day during a four-day interval (60–63rd day) was marked by a pronounced *increase* in the output of allantoin. This point receives further consideration in a later paragraph.

TABLE III.—ALLANTOIN AND PURINE EXCRETION.

Dog No. 2.	Daily output.			
	Output of allantoin-nitrogen.		Output of purine-nitrogen.	
Day of expt.	Gram.	Per cent. of total nitrogen.	Gram.	Per cent. of total nitrogen.
		Preliminary Period.		
5–9	0.016	0.19	0.054	0.64
		Fasting Period.		
10	0.025	0.97	0.006	0.23
11	...	....	...	....
12	0.010	0.17	0.039	0.66
13	0.008	0.23	0.011	0.31
14	0.016	0.66	0.005	0.21
15	0.006	0.21	0.004	0.14
16	0.006	0.21	0.003	0.10
17	0.002	0.11	0.003	0.16
18	0.007	0.17	0.007	0.17
19	0.002	0.08	0.005	0.19
20	0.004	0.14	0.003	0.11
21	0.019	0.63	0.007	0.23
22	0.007	0.32	0.008	0.36
23	0.011	0.29	0.012	0.32
24	0.011	0.36	0.020	0.65
25	0.007	0.53	...	....
26	0.017	0.53	0.016	0.50
27	0.020	0.50	0.015	0.37
28	0.020	0.74	0.014	0.52
29	0.003	0.18	0.008	0.48
30	0.019	0.90	0.006	0.28
31	0.019	0.89	0.006	0.28
32	0.020	0.36	0.016	0.29
33	0.016	0.65	0.009	0.36
34	0.021	0.64	0.010	0.31
35	0.016	0.69	0.010	0.43
36	0.019	0.73	0.014	0.54
37	0.019	0.72	0.002	0.08
38	0.024	0.69	0.006	0.17
39	0.015	0.62	0.007	0.29

TABLE III (*Continued*).

Day of expt.	Output of allantoin-nitrogen.		Output of purine-nitrogen.	
	Gram.	Per cent. of total nitrogen.	Gram.	Per cent. of total nitrogen.
40	0.015	0.62	0.007	0.29
41	0.008	0.34	0.006	0.25
42	0.008	0.34	0.006	0.25
43	0.006	0.22	0.005	0.23
44	0.006	0.22	0.005	0.23
45	0.011	0.48	0.006	0.26
46	0.011	0.48	0.006	0.26
47	0.013	0.43	0.005	0.17
48	0.013	0.43	0.005	0.17
49	0.016	0.55	0.005	0.17
50	0.016	0.55	0.005	0.17
51	0.017	0.61	0.006	0.22
52	0.017	0.61	0.006	0.22
53	0.012	0.36	0.007	0.21
54	0.012	0.36	0.007	0.21
55	0.019	0.46	0.014	0.34
56	0.019	0.44	0.014	0.33
57	0.033	0.55	0.008	0.13

Allantoin Excretion of Dog No. 2.—The data from this animal are tabulated in Table III. An examination of this table will indicate that the average daily output of allantoin-nitrogen during the days of normal feeding preceding the fast was 0.016 gram, or 0.19 per cent. of the total nitrogen excretion for that period. With the inception of the fast this value rose on the first day to 0.025 gram, or 0.97 per cent. of the total nitrogen output. This was the second largest output of allantoin-nitrogen secured during the fast, the largest being an output of 0.033 gram which was eliminated upon the very last day of the experiment. Although the output of 0.025 gram excreted upon the first fasting day was not so large as that secured upon the 48th day of fast, it did, nevertheless, constitute a greater proportion of the total nitrogen than did the 48th day excretion of 0.033 gram. The latter value was only 0.55 per cent. of the total nitrogen eliminated on that day, whereas the allantoin quota for the first day constituted 0.97 per cent. of the total nitrogen output, as already mentioned.

In general, the allantoin-nitrogen elimination was less during the early part of the fasting period than it was at later stages in the fast. For example, the average daily excretion for the first third of the fast (16 days) was 0.009 gram, for the second third 0.017 gram, and for the remainder 0.014. It will be observed that the daily average excretion for the first part of the fast was much lower than that of the preliminary feeding period, whereas the daily average for the remainder of the fast was very close to the feeding level. The grand average excretion per

day for the entire 48-day fast was 0.013 gram as compared with an output of 0.016 gram for the preliminary period. There was therefore an actual decrease in the output of allantoin as a result of the fast; the proportion of the total nitrogen of the fasting period which was made up by allantoin-nitrogen was, however, markedly increased. This was increased between 200 per cent. and 300 per cent. during the fast above that in force during normal feeding.

TABLE IV.—ALLANTOIN AND PURINE EXCRETION.

Dog No. 6.	Daily averages.					
	Allantoin-nitrogen.			Purine-nitrogen.		
Day of expt.	Total. Gram.	Daily average. Gram.	Per cent. of total nitrogen.	Total. Gram.	Daily average. Gram.	Per cent. of total nitrogen.
		Preliminary Period.				
7–9	0.001	0.0004	0.32	0.002	0.0007	0.65
10–12	0.002	0.0007	0.35	0.003	0.0010	0.52
Period av.		0.0005	0.34		0.0008	0.56
		Fasting Period.				
13–16	0.004	0.0010	0.17	0.007	0.002	0.29
17–19	0.002	0.0007	0.11	0.006	0.002	0.34
Period av.		0.0009	0.145		0.002	0.31

Allantoin Excretion of Dog No. 6.—The data for this dog are given in Table IV. If this table be examined, it will be noted that the average daily excretion from the 7th to 9th day of the preliminary period was 0.0004 gram or 0.32 per cent. of the total nitrogen output. From the 10th to 12th day of this same period the average daily output was 0.0007 gram, or 0.35 per cent. of the total nitrogen. The grand average daily excretion for the whole preliminary period was 0.0005 gram, or 0.34 per cent. of the total nitrogen excreted during this interval.

The fast continued for a period of one week. During the first four days (13–16) the daily output of allantoin-nitrogen was 0.0010 gram, a value double the daily average of the preliminary period. On the three following days, the output fell somewhat but still remained above that for the preliminary period. The average output for these days was 0.0007 gram. Taking into consideration the entire fast of 7 days the grand average daily output of allantoin-nitrogen was 0.0009 gram, a value which was very nearly double that of the preliminary period.

Notwithstanding the fact that the actual quantity of allantoin-nitrogen eliminated per day was much higher during the fasting interval than during the days of normal feeding, it is a significant fact that the proportion of the total nitrogen output which the allantoin represented was less than one-half as great as it had been previous to the opening of the fast. The table shows that the allantoin accounted for 0.34 per cent. of the total nitrogen during the preliminary period, whereas 0.145 per cent. of the total nitrogen was made up by the allantoin during the fasting inter-

val. This is a much more striking reversal in the course of the allantoin excretion in the change from normal feeding to a fasting régime than was noted in the case of either of the dogs previously considered.

Comparison of Allantoin-nitrogen Data.—A brief comparison of the data from the analysis of the urine of the three dogs, as well as that of the fourth dog previously mentioned, will be of interest. Of the four dogs under consideration three were adults, dog No. 1 being the youngest of the three (1–2 years of age), whereas one dog (No. 6) was a pup only one month old. We may consider then that we have two distinct types of animal so far as age is concerned.

First let us compare the data from the adults. dog No. 3 exhibited a decreased excretion of allantoin under the influence of the fast. However, when we come to examin into the question of the proportion of the total nitrogen elimination, which this allantoin-nitrogen represents, we see that the percentage underwent a pronounced rise during the fasting interval.

In the case of dog. No. 2 we again observe an actual decrease in the average daily output of allantoin-nitrogen as we change from a feeding to a fasting régime. The decrease is, however, much less pronounced than in the case of dog No. 3. In fact, during the latter $^2/_3$ of the fast the daily output is very nearly equivalent to that of the feeding level. When we consider the relation between total nitrogen and that in the form of allantoin we find a condition similar to that in force with dog No. 3. In other words, the allantoin excretion of the fasting days made up a larger quota of the total nitrogen output than it did during the feeding interval. The difference in this respect was much more marked in the case of dog No. 2 than in the case of dog No. 3.

TABLE V.—ALLANTOIN AND PURINE EXCRETION.

Dog No. 1. Daily averages.

Period.	Length in days.	Purine-nitrogen. Gram.	Purine-nitrogen. Per cent. of total nitrogen.	Allantoin-nitrogen. Gram.	Allantoin-nitrogen. Per cent. of total nitrogen.
Preliminary	8	0.012	0.45	0.008	0.31
First fast	15	0.005	0.22	0.005	0.26
Preliminary feeding	4	0.006	0.45	0.009	0.71
Normal feeding	15	0.006	0.35	0.005	0.28
50 per cent. increase in diet.	8	0.007	0.27	0.013	0.50
100 per cent. increase in diet.	17	0.015	0.40	0.006	0.15
Normal feeding	3	0.008	0.33	0.005	0.20
Second fast	30	0.005	0.38	0.004	0.31

If we examin the data for dog No. 1, as given in Table V, we will observe that the course of the allantoin-nitrogen excretion was very similar to that secured from the analysis of the urine of dogs Nos. 2 and 3, so far as the actual average daily output of this form of nitrogen was concerned.

For example, it will be noted that the average daily output of allantoin-nitrogen for the first fast (15 days) to which this animal was subjected was considerably lower than the excretion of the preliminary period calculated upon the same basis. The ratio in this instance is 5 : 8. Again taking the second fast, we here find that in this instance the fasting level of the allantoin-nitrogen elimination is somewhat lower than the feeding plane. The ratio is less pronounced than that in force during the first fast, however, being 4 : 5.

If we consider the percentage of the total nitrogen output of dog No. 1, which was eliminated in the form of allantoin-nitrogen, we find a condition of affairs entirely different from that which was obtained with dogs Nos. 2 and 3. In the first fast of dog No. 1, for example, the allantoin-nitrogen constituted 0.26 per cent. of the total nitrogen whereas this form of nitrogen composed 0.31 per cent. of the total nitrogen excreted during the period of normal feeding. Dogs Nos. 2 and 3, it will be remembered, each exhibited during the fast a very pronounced increase above the feeding value in the percentage of the total nitrogen which appeared as allantoin-nitrogen. The data from dog No. 1, however, show a decreased percentage excretion of allantoin-nitrogen as we pass from feeding to fasting. In other words, the course of the percentage allantoin output for this animal during the first fast exhibits the characteristics of the percentage allantoin output of the pup (dog No. 6). The decrease in the percentage values, however, was much sharper in the case of the pup. When we turn to the second fast of dog No. 1 we find that the conditions regarding the percentage elimination of allantoin-nitrogen are reversed and are rather more in keeping with those from the adult dogs Nos. 2 and 3. The increase in the percentage values during this second fast is still much less, however, than that previously observed in the case of these two animals.

It seems, therefore, when we take into account the allantoin values from the four dogs in question that there are pronounced variations in the actual output of allantoin as well as in the ratio which this output bears to the total quantity of nitrogen excreted per day. With dog No. 3 the change in the course of the allantoin excretion as we passed from feeding to fasting consisted in a pronounced *decrease in the quantity of allantoin actually excreted*, accompanied by a much more pronounced *increase in the percentage relationship* between this allantoin output and that of total nitrogen. With dog No. 2, while there was a decrease in the actual quantity of allantoin excreted per day, this decrease was small as compared with that observed in connection with dog No. 3. The increase in the percentage values was rather more decided, however, in this fasting animal than in No. 3. In opposition to these findings, we observe when we turn to the pup that the fasting period yields us an *increase in*

the actual quantity of allantoin-nitrogen excreted per day and at the same time furnishes a *decrease in the percentage of the total nitrogen* which this allantoin-nitrogen represents. Here, then, we have a decided contrast in the course of the allantoin excretion of the adult dogs as compared with that of the animal but lately weaned. The course of the allantoin excretion of dog No. 1 apparently stands midway between those of the adult animals 2 and 3 and the pup (6). We have a decrease in the output of allantoin-nitrogen during the fast as compared with the feeding level, a feature which is held in common by adult dogs Nos. 2 and 3. The decrease is, however, much less pronounced than that observed in the data for 3. The percentage of the total nitrogen which is excreted as allantoin undergoes a *decrease* as a result of the first fast, a finding in accord with the data from the pup and directly opposit to the percentage values for the allantoin excretion of the adult dogs. As we pass from normal feeding to the second fast an *increase* in the percentage values is observed, but this increase is in no way comparable to the more marked increase which was in force during the fasts of dogs Nos. 2 and 3. It is an interesting and very significant finding that this animal, which stood in an intermediate position between the adult dogs and the pup as regards age, should yield allantion values which also occupy an intermediate position between the allantoin values furnished by the other dogs in question.

Purine Excretion. Purine Excretion of Dog No. 3.—The data from the purine excretion of this animal are given in Table II. It will be observed that the average daily output of purine-nitrogen during the preliminary period was 0.067 gram, or 0.29 per cent. of the total nitrogen excreted. On the first day of the fast the actual amount of this form of nitrogen which was eliminated in the urine rose to 0.098 gram, a value which constituted 1.05 per cent. of the total nitrogen excreted during that 24-hour interval. At no time in the remaining 95 days of the fast did these values approach those secured upon the first day. This very marked increase in the purine excretion upon the first fasting day is a very significant finding.

There was no very decided regularity in the average daily output of purine nitrogen during the fast, although the average was decidedly lower during the second half of the fast than it was during the first half. The average percentage values were also lower during the later stages of the fast than they were during the earlier stages, although the variation in this respect was not very marked. Under the influence of a pronounced increase in the water ingestion, the purine-nitrogen excretion underwent a profound *decrease* from the 60th to the 63rd day, inclusive. This feature is further discussed in a later paragraph.

Purine Excretion of Dog No. 2.—For data regarding this subject see Table III. These data indicate that the average daily output of purine-

nitrogen during the period of normal feeding was 0.054 gram or 0.64 per cent. of the total nitrogen value. On the first day of the fasting period these values were subjected to a profound alteration. It will be noted that the actual quantity of purine-nitrogen excreted on this day amounted to but 0.006 gram, a weight which was equivalent to only 0.23 per cent. of the total nitrogen output for that 24-hour interval. This pronounced lowering of these values upon the first fasting day is in marked contrast with the first-day findings for the other adult dog (3). With this dog (3), as already mentioned, the actual quantity of purine-nitrogen eliminated upon this day as well as its percentage relationship to the total nitrogen excretion both underwent a very *decisive increase*, whereas in the case of dog No. 2 the result was a *decrease* which was fully as marked as the increase had been in the case of dog No. 3.

The maximum fasting excretion (0.039 gram) for dog No. 2 occurred on the 3rd day of the fast. This value also made up a larger quota (0.66 per cent.) of the total nitrogen than did the output for any other day of the fast. The average daily output of purine-nitrogen for the first part of the fast was rather higher than that for the second part. This was true in spite of the fact that the last three days of the fasting interval yielded a higher average daily output than was secured for a period of nearly four weeks previously. If we divide the fast into three 16-day periods we find that the average daily output of purine-nitrogen was uniform during the first $^2/_3$ of the fast and underwent a decided decrease in the final interval. The ratio existing between these three intervals is 10 : 10 : 7. In other words, the average output of purine-nitrogen was uniform for a fasting interval of 32 days, at which time it underwent a drop of 30 per cent.

Purine Excretion of Dog No. 6.—In Table IV will be found the purine-nitrogen data from this animal. It will be observed that the average daily output of this form of nitrogen was 0.0007 gram, or 0.65 per cent. of the total nitrogen during the 7th, 8th, and 9th days and that this value was increased to 0.0010 gram, or 0.52 per cent. of the total nitrogen during the 10th, 11th, and 12th days. The grand average daily elimination of purine-nitrogen for this period of normal feeding was therefore 0.0008 gram, or 0.56 per cent. of the total nitrogen.

When the fast opened the daily average fell to 0.002 gram and maintained that level uniformly throughout the entire fast. The percentage of the total nitrogen which was excreted in the form of purine-nitrogen was also very uniform, ranging from 0.29 per cent. to 0.34 per cent. with a grand average of 0.31 per cent.

Comparison of Purine-nitrogen Data.—In the comparison of the allantoin-nitrogen data given in a previous paragraph, it was pointed out that there were very marked differences in the course of this excretion

observable in connection with the different animals. These variations seemed, to a certain degree, to be functions of the age of the animal. For example, the course of the allantoin-nitrogen excretion of the month-old pup was widely divergent from that of the adult dogs and furthermore the dog (No. 1) which occupied an intermediate position between the adults on the one hand and the pup on the other, so far as age was concerned, exhibited this same intermediate relationship in respect to allantoin excretion.

When we come to consider the purine-nitrogen data from the comparative view point we find no such relationship as that noted for allantoin. It will be observed, for example, that adult dog No. 2, in passing from the feeding period to the fast, exhibited a *decrease* in both the actual quantity of purine-nitrogen excreted per day and in the percentage of the total nitrogen output which this represented. If we now compare these findings with the data from the pup we find a *similar relationship*, *i. e.*, a decrease in both the actual output and the percentage value. The data from dog No. 1 show a similar relationship during her first fast, whereas the percentage values were slightly increased during the second fast. When we turn to the data on the purine-nitrogen output of dog No. 3 we find for the first time an important variation from the uniform relations which the purine data have thus far shown. Here we note the same decrease in the actual quantity of purine-nitrogen eliminated per day, but a consideration of the percentage data reveals for the first time an increase in the percentage of the total nitrogen output, which this form of nitrogen comprizes. This increase is approximately 30 per cent. above the value for the preliminary period. It is worthy of note that this increase would have been greater had the purine-nitrogen output not been subjected to a marked diminution during the 60–63rd days, through the influence of a high water intake. This feature of the investigation is discussed in full in the next section.

Influence of a Pronounced Increase in the Water Ingestion of a Fasting Animal upon the Allantoin and Purine Output.—It will be observed upon examining Table II that the daily output of allantoin as well as the percentage of the total nitrogen which is excreted in this form both undergo a sharp rise during the period represented between the 60th and 63rd days, inclusive. This rise in the allantoin output is not due to fasting. Up to this time the dog had been receiving 700 cc. of water per day by means of a stomach tube and during the period in question (60–63rd days) this water ration was increased 200 per cent. It was evidently due to the introduction of this extra volume of water that the heightened allantoin output appeared. Previous to the high water intake the animal had been excreting on an average 0.011 gram of allantoin-nitrogen per day, or 0.36 per cent. of the total nitrogen output. The influence of this

2100 cc. of water was observed at once, inasmuch as an allantoin-nitrogen output of 0.036 gram, or 0.71 per cent. of the total nitrogen excretion, was secured upon the first day of its ingestion. The allantoin output increased still further and upon the third and fourth days of this copious water ingestion it was equivalent to 1.16 per cent. and 1.00 per cent. of the total amount of nitrogen eliminated. The high values for the allantoin output disappeared as soon as the original level of water ingestion was again assumed.

How is this marked increase in allantoin output during the period of copious water intake to be explained? If we examin the purine data for this same period we will note that there was a pronounced diminution in the output of purine-nitrogen during the days upon which the allantoin-nitrogen was undergoing the striking increase already mentioned. For forty days previous to the period of high water ingestion the dog had been eliminating from 0.013 to 0.016 gram, or 0.41 to 0.56 per cent. of the total nitrogen in the form of purine-nitrogen. At the opening of the water period these values dropped to 0.005 gram, or 0.10 per cent., at which point the purine output remained uniform for two days, then underwent a further decrease to 0.002 gram, or 0.07 per cent., a level which was maintained for the remainder of the water period. There was therefore a very marked *decrease* in the output of purine-nitrogen during the water period, and coincidently with this lowered output of purine-nitrogen comes the pronounced *increase* in the allantoin-nitrogen excretion. In the light of our present knowledge as to the metabolic relationship of purines and allantoin there is apparently but one interpretation to be placed upon these findings. It has been shown by experimentation that purine bodies may be oxidized with the resultant formation of allantoin. It has furthermore been demonstrated that the allantoin output may be increased by purine feeding. It seems clear then that in the present instance the increase in the allantoin excretion and the associated decrease in the purine output are due to the fact that the large volume of water (2100 cc.) fed the dog on each of the days of the water period has stimulated the oxidative processes of the organism to a profound degree. Through the medium of this increased oxidation such substances as would ordinarily be eliminated in the urine and go to increase the purine-nitrogen output have been oxidized to allantoin and appear in the urine in this form. An examination of the data will show that the oxidation of the nitrogen of purine origin into allantoin was almost quantitative in the final days of the water period. For example, on the 62nd day of the experiment the total elimination of nitrogen of purine origin was 1.23 per cent., of which all but 0.07 per cent. was in the form of allantoin. Again, on the 63rd day, with a total output of 1.07 per cent. of nitrogen of purine origin, all but 0.07 per cent. of this nitrogen was

excreted as allantoin. The purine-nitrogen output was still low for one day immediately after the water period, but from this point on to the end of the experiment the figures for this type of nitrogen were more nearly comparable to those secured during the period previous to the interval of high water ingestion.

The allantoin and purine data of the water period are of further interest in connection with the output of uric acid under the influence of copious water drinking. In another paper from this laboratory[1] it was shown that in man a *decreased* output of uric acid accompanied copious water drinking. Inasmuch as the Folin method for the determination of this urinary constituent had been shown to be rather inaccurate when used to determin the uric acid content of very dilute urines no particular significance was attached to the data which indicated a lowered uric acid output under the influence of water drinking. However, if we take our allantoin and purine findings into account in this connection, we have a very plausible explanation for the phenomenon of a lowered uric acid excretion under such conditions. Evidently when water in large quantity is ingested the nuclein material which gives rise to the uric acid of the urine is subjected to a more profound oxidation than under the régime of a low water ration. Through the medium of this augmented oxidation a certain quota of the uric acid is oxidized and may appear in the urine as allantoin. If this is true, then we would obtain a lowered output of uric acid under the influence of copious water drinking. In the work already referred to as having been done in this laboratory it may very well be that the inaccuracy in method may not be entirely responsible for the low uric acid values obtained during the days of high water ingestion. There may have been an *actual decrease* in the quantity of uric acid eliminated upon those days, due to its oxidation into allantoin. The contention of Schöndorf[2] and of Genth,[3] who claim to have demonstrated a lowered output of uric acid after water drinking, may be worthy of consideration, although their methods were rather open to criticism. On the other hand, if water does cause this increased oxidation of uric acid into allantoin it is hard to reconcile the findings of Laquer[4] and of Schreiber,[5] each of whom maintains that the uric acid output is slightly *increased* under the influence of an increased water ration. Of interest in this connection are the findings of Beebe[6] in his study of the influence of alcohol upon the uric acid output. The ingestion of 50 cc. of absolute alcohol (diluted to 200 cc. with water) by a man caused the hourly urine

[1] Rulon and Hawk, THIS JOURNAL, **32**, 1686 (1910).
[2] *Arch. ges. Physiol.*, **46**, 529.
[3] Quoted by Schöndorf, *Arch. ges. Physiol.*, **46**, 529.
[4] Kongress für innere Medicin, **1896**, p. 381.
[5] Schreiber, "Die Harnsaüre," **1899**, p. 38.
[6] *Am. J. Physiol.*, **12**, 120 (1904).

volume to increase from 110 cc. to 465 cc. and then to 565 cc., the volume thereafter dropping abruptly to 57 cc. and 35 cc. during the next two one-hour intervals. Accompanying this diuresis a *pronounced decrease* in the output of uric acid was noted. The author says "Sweeping urates out of the system by increasing the volume of water excreted finds little support in this experiment." Mendel and Brown[1] also failed to find any correlation between an increased urine flow and an augmented uric acid output.

It is a significant fact that the total output of nitrogen of purine origin,[2] *i. e.*, purine-nitrogen plus allantoin-nitrogen, was *increased* during the period of high water intake. For forty days previous to the commencement of the water period the total daily output of nitrogen of purine origin had ranged from 0.007 to 0.026 gram, the average for the forty days being 0.021 gram. With the opening of the water period the average of 0.021 gram was increased to 0.041 gram, whereas values of 0.028 gram, 0.038 gram and 0.030 gram were obtained for the remaining days of the period, all of them being considerably above the pre-water average. The daily average for the water period was 0.034 gram as against an average of 0.021 gram for the forty days immediately preceding this period, and 0.019 gram for the 33 days following the period. It is thus evident that there was a definit increase in the total quantity of nitrogen of purine origin which was excreted per day during the period of high water intake. We would interpret this finding as indicating that the water caused a stimulation of the catabolism of muscular tissue and a consequent increase in the output of total purine (allantoin + purine).

The total nitrogen data from this animal show that there was an average daily elimination of 2.872 grams of nitrogen for 6 days previous to the water period, an output which was augmented to an average excretion of 3.669 grams under the influence of water. There was therefore an actual increase of 3.188 grams of nitrogen during the four-day period in which the animal received 2100 cc. of water per day. If we express this nitrogen value in terms of flesh we find it is equivalent to 98 grams, and if we go still further and calculate the amount of purine nitrogen which this weight of flesh should yield if catabolized, we learn that this is 0.059 gram of pure nitrogen. We can readily determin from an examination of the tabulated data that the actual increase in the output of total purine-nitrogen as determined during the water period was 0.032

[1] *J. Am. Med. Assoc.*, **49**, 896 (1907).

[2] Professor F. G. Benedict tells us he recalls having read some years ago an article on water drinking which recorded an increased output of nitrogen of purine origin. However, neither Professor Benedict nor ourselves have been able to locate the reference.

gram. In other words, if 98 grams of flesh were catabolized we cannot account for 46 per cent. of its contained purine-nitrogen. This portion of the purine-nitrogen may have been oxidized to stages below allantoin and thus escaped detection. The calculations mentioned above are summarized below:

RELATION BETWEEN TOTAL NITROGEN AND TOTAL PURINE NITROGEN (ALLANTOIN + PURINE) EXCRETIONS UNDER INFLUENCE OF WATER.

Average daily elimination of total nitrogen[1] for 6 days previous to water period	2.872	grams
Average daily elimination for water period	3.669	"
Daily increase in total nitrogen output during water period	0.797	"
Total increase in total nitrogen output during water period	3.188	"
Equivalent in terms of flesh (3.25 per cent.)	98.0	"
Percentage of purine-nitrogen in flesh	0.060	per cent.[2]
Average daily ouput of total purine-nitrogen for 10 days preceding water period	0.026	gram
Average daily output during water period	0.034	"
Total increase during the 4-day water period	0.032	"
Purine-nitrogen in 98 grams of flesh	0.059	"
Purine-nitrogen unaccounted for	0.027	" or
	46.0	per cent.

The two theories which have been advanced by different investigators to account for the increased nitrogen output which accompanies water drinking have been fully discussed in previous papers from this laboratory.[3] In the paper by Fowler and Hawk were presented data which appeared to furnish, for the first time, conclusive evidence in substantiation of the theory that this increased nitrogen output was due, at least in part, to the catabolism of muscular tissue. The appearance of creatine in the urine during the water period was believed to furnish this evidence. We now submit our total purine data as further substantiation for this same theory. As far as we can see, the total purine nitrogen output of this fasting dog could have been increased during the water period only through the influence of the water in stimulating protein catabolism. To be sure when we come to correlate the total nitrogen data with the total purine nitrogen data we can account for only about one-half of the total nitrogen output on the basis of our purine figures. However, we could logically hardly expect a quantitative result here when we take into account the character of the method and the further fact that the allantoin output may very well have been lowered through the subsequent oxidation of a certain portion of this substance into other undetermined nitrogenous substances.

[1] These data will be published in a forthcoming article.

[2] Hall, "The Purine Bodies in Foodstuffs," Manchester, 1902, p. 29, Table IV.

[3] Hawk, Univ. of Penn., *Medical Bulletin* 18, 7 (1905). Fowler and Hawk, *J. Exp. Med.*, 12, 388 (1910). Wills and Hawk, unpublished.

In any attempt to show a mathematical relationship between the urinary total purine nitrogen and the total nitrogen output as given in an earlier paragraph, it must be borne in mind that the flesh of the different portions of the same animal will vary in their purine values. For example, Hall[1] has shown that pork loin may contain 0.048 per cent. of purine-nitrogen, whereas the neck may contain but 0.023 per cent. At the same time the neck cut of a calf may contain 0.030 per cent. while the loin contains 0.071 per cent.[2] The purine values of ox flesh have been placed at 0.062 per cent. and 0.063 per cent., whereas horse flesh has been found to yield 0.055 per cent. of purine-nitrogen.[3] It is without doubt true that any factor which stimulates protein catabolism through the medium of the circulation, as does water, must of necessity stimulate this process in all parts of the organism, the stimulatory influence being greatest in those portions which have the most plentiful blood supply. In the case in point, therefore, with our fasting dog, the increased output of total purine nitrogen has no doubt resulted from the stimulated catabolism of tissues which have ranged in purine values from 0.030 to 0.063 per cent. at least. There is obviously no possible way of estimating what percentage of this increased output is due to the disintegration of the one type of tissue as distinct from the others. If we were to make our calculations on the basis of the 0.030 per cent. value, it would be possible to show an almost quantitative relationship between the excretion of total purine-nitrogen and total nitrogen. Such a calculation could, however, have but slight significance. We have therefore taken 0.060 as the basis of our calculations, for the reasons that the portions of the animal which yield such low purine values as 0.030 form in all cases but a minimum portion of the total weight of the carcass. This value of 0.060 per cent. is also suggested by Burian and Hall[1] as a satisfactory average for the purine-nitrogen value of flesh.

Summary.

The experiments here reported embraced the study of the course of the allantoin-nitrogen and purine-nitrogen excretion of four dogs which were subjected to fasting periods ranging in length from one week to 96 days. Water was fed by means of a stomach tube in uniform quantity from day to day during the fast, the volume being regulated according to the body weight of the animal. The adult dogs fasted from 48 to 96 days, whereas a pup one month of age was the subject of the 7-day fast.

When the data for the allantoin-nitrogen excretion of the various dogs are compared, pronounced variations are noted between the different dogs and these variations appear to be functions of the age and develop-

[1] *Loc. cit.*

[2] Burian and Hall, *Z. physiol. Chem.*, **38**, 336 (1903).

[3] Burian and Hall, *Loc. cit.*

ment of the animals in question. For example, in the case of the adult animals we have a *decreased* output of allantoin-nitrogen when the feeding period gives way to a fasting régime, whereas in the case of the pup the allantoin values are nearly *double during the fasting interval* over those secured during the period of normal feeding. Likewise, when we turn to a consideration of the percentage relationship we find that the percentage of the total nitrogen which is excreted as allantoin is *increased* in the case of the adult dogs and *decreased* in the case of the pup. When the data secured from the adult dogs and the pup are compared with those secured from a dog midway between these two types of animal, so far as age is concerned, we find that the course of the allantoin-nitrogen values was also of an intermediate character.

The output of purine-nitrogen does not show the variation noted in connection with the allantoin-nitrogen. There was a well defined tendency toward a *decreased excretion* of purine-nitrogen as well as a *decreased percentage relationship* as the well-nourished animals were subjected to the withdrawal of food. In other words, during the fast there was, in general, a lower elimination of allantoin-nitrogen than during the feeding period and this lowered elimination also constituted a smaller percentage of the total nitrogen output. This percentage relationship did not hold for one dog which fasted 96 days and exhibited an increased percentage relationship during the fast above that in force during the period of normal feeding.

The question of the influence of a *pronounced increase in the water ingestion of a fasting animal* upon the allantoin and purine excretion was studied upon one adult dog. This dog received 700 cc. of water daily for 59 fasting days and for a period of four days from this time the water ingestion was increased to 2100 cc. per day. This high water ingestion produced a *marked increase in the output of allantoin-nitrogen* and a much more *pronounced decrease in the purine-nitrogen* output. These findings were interpreted as indicating that the high water ingestion had caused sufficient stimulation of the oxidative processes of the body to bring about the oxidation into allantoin of such substances as would ordinarily go to make up the purine-nitrogen quota. This oxidation was almost quantitative during the latter part of the water period. During this interval only 0.07 per cent. of the nitrogen of purine origin was excreted in the form of purine-nitrogen. The finding of a lowered output of purine nitrogen was in full agreement with the finding already reported from this laboratory of a lowered output of uric acid under the influence of copious water drinking.

It was further found that the *total purine* values (sum of the allantoin-nitrogen and purine-nitrogen) for the four days of increased water ingestion were higher than the total purine values for any other four-day

period during the fast. This finding was interpreted as indicating that the high water intake had caused a stimulation of protein catabolism. The total nitrogen was also increased. Knowing the percentage of purine-nitrogen in flesh and the percentage of total nitrogen in flesh a relationship was established between the output of purine nitrogen and the total nitrogen excretion. By this means it was learned that, provided sufficient flesh was catabolized to yield the increased output of total nitrogen eliminated during the water period, 46 per cent. of the purine-nitrogen of this quantity of flesh was unaccounted for. This quota of purine-nitrogen might have been oxidized to stages below allantoin and thus escaped determination.

We submit the data showing an increased output of total purine-nitrogen (allantoin-nitrogen + purine-nitrogen) under the influence of a high water ingestion as evidence in favor of the hypothesis that the water stimulated protein catabolism.

URBANA, ILL.

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY OF HARVARD MEDICAL SCHOOL.]

THE DETERMINATION OF BENZOIC ACID.

BY OTTO FOLIN AND FRED F. FLANDERS.

Received August 4, 1911.

A recent investigation on cranberries undertaken in this laboratory involved a great many determinations of benzoic acid. Although methods for benzoic acid have undergone much improvement recently, it was soon evident that a great deal of time would be consumed in carrying out the large number of determinations which the work demanded.

The method first used was the second modification of the LaWall and Bradshaw method, described in Bulletin 132 of the Bureau of Chemistry.[1] Considerable experience had previously been had by one of us[2] with this method, and its weak points were well known. The filtration of the alkaline solution is usually very tedious and often nearly impossible. The extraction of the acidified filtrate is slow and difficult, as the tendency to form an emulsion is very great. After the extraction the chloroform must be allowed to evaporate and the residue dried in a desiccator to remove acetic acid, which requires much time. Finally the titration in alcoholic solution with phenolphthalein as indicator is open to criticism. This latter objection has been in some degree overcome by Clark[3] by adding water and titrating directly in the chloroform with aqueous alkali.

It occurred to us that this titration might be better made in the chloroform directly, according to the principle recently advanced by Folin and

[1] U. S. Dept. Agr., Bur. of Chem., *Bull.* 132, p. 140 (method 2).

[2] Flanders.

[3] U. S. Dept. Agr., Bur. of Chem., *Bull.* 132, p. 147.