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STUDIES ON WATER DRINKING. XII. ON THE ALLANTOIN OUTPUT OF MAN AS INFLUENCED BY WATER INGESTION.[1]

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Introduction.

The allantoin literature was reviewed in a recent article from this laboratory.[3] Very little work on allantoin has appeared in the scientific press since that time. The recent work of Hunter and Givens[4] is of particular interest. They used monkeys as subjects and isolated the allantoin from the urin of the animal by the method of Wiechowski. They could detect no uric acid and found only 4.5 mg. of purine base nitrogen in 500 cc. of urin. The same authors have just reported further findings on the normal purine metabolism of the monkey.[5] They found that 71-87% of the total purine nitrogen appeared as allantoin. When sodium nucleate was fed, sometimes as much as 90% of it was recovered, for the most part as allantoin. It is evident, therefore, that the monkey possesses a uricolytic enzyme which splits uric acid into allantoin. An analogous condition has not, thus far, been definitely demonstrated for the human organism. Schittenhelm and Wiener,[6] for example, after feeding nuclear material failed to observe what they considered to be an increased output of allantoin. The allantoin output in these instances was small, to be sure, but nevertheless the authors mentioned present no data from control periods for comparison. We cannot be certain, therefore, that the allantoin output, though small, *was not increased* under the influence of the feeding of the nuclein-bearing material. Moreover, the subjects used were clinical patients and it is quite possible that their metabolic attitude toward the ingested material was not that of a normal organism. The finding by Ascher[7] of allantoin in the urin of a fasting man indicates that in the human organism, as well as in that of the lower animals, allantoin may have an endogenous origin. Objections have been raised to such an interpretation on the basis that the fasting period in Ascher's experiment was not long enough to insure the non-influence of the feeding period upon the allantoin output.

[1] Reported before Am. Soc. Biol. Chem., Baltimore, December, 1911.

[2] The authors wish to thank Mr. L. A. Fritze for assistance rendered during the early stages of the allantoin determinations.

[3] Wreath and Hawk, THIS JOURNAL, 33, 1601 (1911).

[4] *Am. J. Physiol.*, 27, 1911.

[5] Reported before Soc. Biol. Chem. and Biological Section Am. Chem. Soc., Washington, December, 1911.

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

[7] *Biochem. Z.*, 26, 370 (1910).

ALLANTOIN EXCRETION AS INFLUENCED BY WATER INGESTION.

Days.	Water ingested. Cc.	Allantoin excreted Gram.
Fore period:		
13	900	0.0135
Water period:		
1	3450	0.0173
2	3450	0.0166
3	3450	0.0157
4	3450	0.0162
5	3450	0.0205
Average		0.0173
Final period:		
5	900	0.0122

Certian perfusion experiments of Ackroyd[1] are of interest. This investigator found that when sodium urate was perfused through the surviving liver of the dog that a portion of the urate was destroyed. Only part of the destroyed urate was recovered as allantoin. In later experiments of this character in which rabbits were used as subjects, it was found that the urate was converted quantitatively into allantoin.[2] Ackroyd has examined certain foods for allantoin, and reports that milk, white bread, French peas and green peas all contain allantoin in small amount, whereas more could be detected in eggs, bananas or rhubarb.[3] The failure of Schittenhelm and Wiener[4] to find any allantoin in the urin of a subject maintained upon a diet of milk and vegetables permits of the query as to whether the urinary allantoin excretion is influenced by the allantoin which forms a constituent part of the ingested food. This question has been partly answered by further work of Schittenhelm and Wiener,[5] in which it was shown that only 30% of the allantoin ingested by human subjects in the form of an aqueous solution was recoverable in the urin.

Experimental.

It was proposed to demonstrate what influence, if any, would be exerted upon the urinary allantoin output of a normal man when he was caused to ingest large quantities of distilled water at meal time. The subject of the experiment was a man weighing 57.5 kg. The plan of the investigation was the same as that utilized in this laboratory in connection with previous water-drinking researches.[6] The experiment was

[1] *Biochem. J.*, **5**, 217 (1910).
[2] *Ibid.*, **5**, 442 (1911).
[3] *Ibid.*, **5**, 400 (1911).
[4] *Loc. cit.*
[5] *Z. physiol. Chem.*, **63**, 287 (1909).
[6] Fowler and Hawk, *J. Exp. Med.*, **12**, 388 (1910). Mattill and Hawk, THIS JOURNAL, **33**, 1978 (1911); *Ibid.*, p. 1999; *Ibid.*, p. 2019.

divided into three separate periods, a fore or control period, a water period and a final period. The following uniform diet was fed each day throughout the experiment: 300 grams graham crackers, 1200 grams whole milk, 75 grams butter, 45 grams peanut butter. Thirteen days were included in the fore period and five days in the final period, and during each of these days 900 cc. of water were ingested, 300 cc. of it being taken at meal time. The water period was five days in length and on each day of this period 3450 cc. of water were taken, 2850 cc. of this volume being taken at meal time.

The method used for the determination of allantoin was that proposed by Wiechowski.[1]

Discussion.—The analytical data are summarized in the table on page 547. It will be noted that the average daily excretion of allantoin for the thirteen normal days preceding the interval of high-water ingestion was 0.0135 gram. Upon the first day of the water period when the ingestion of water was increased from 900 cc. per day to 3450 cc., the allantoin output rose to 0.0173 gram and remained above normal throughout the five days of copious water-drinking. The values for the remaining four days were 0.0166, 0.0157, 0.0162 and 0.0205 gram, respectively. The average daily allantoin excretion for the entire water period was 0.0173 gram, which happens to be the identical allantoin value as determined for the initial day of the period. This constitutes an increase of over 20% above the normal allantoin level. At the opening of the final period, during which the water ingestion was reduced to the normal quantity, the daily allantoin output decreased very perceptibly from that observed during the water interval. The average daily output for this final period of five days was 0.0122 gram. It will be observed that this is a decrease of about 30% from the values determined for the water period. It is also somewhat lower than the value as determined for the fore period. This same compensatory effect has been noted in our water experiments in other connections. In other words, when a certain urinary constituent is increased under the influence of a high water ingestion it very often happens that the output of this constituent is sub-normal in the succeeding period. On the other hand, if the constituent has been excreted in sub-normal amount during the water period the post-water interval will many times witness an augmented excretion.

We realize fully that we are dealing here with very small quantities of allantoin. However, inasmuch as we were feeding our subject a diet which was absolutely uniform from day to day we feel justified in concluding that the water, when given in increased quantity, caused an augmentation in the daily allantoin output. We would interpret this find-

[1] Wiechowski, *Biochem. Z.*, 19, 368 (1909).

ing, tentatively, as indicating that the oxidative functions of the organism have been stimulated through the passage of this large volume of water into the organism in question and consequently material of purine origin which would under ordinary conditions have been excreted in other less highly oxidized forms was oxidized to allantoin and eliminated as such (see Summary). In support of this interpretation, we would cite certain other findings reported from this laboratory. In the experiments in question[1] the uric acid output of a normal man living on a normal uniform diet was found to undergo a pronounced decrease when large quantities of water were ingested. At the time this finding was reported, we found it difficult to see "how an increased water ingestion could be considered as a logical forerunner of a *decreased* uric acid excretion." When an *increased* allantoin output was subsequently obtained in an experiment entirely similar in character and also made upon a human subject, the possibility of the interrelationship of these two findings suggested itself to us. It should be mentioned that in the work on uric acid elimination, to which reference has just been made, a large part of the decrease in the output of uric acid was believed to be due to the fact that the method employed (Folin) for its determination was not entirely satisfactory for use in connection with the *dilute* urines of the water period.

Several arguments may be advanced against the validity of our interpretation. In the first place, Ackroyd's[2] demonstration that various food products contain allantoin may be considered by some to eliminate the possibility of interpreting our increased allantoin as of endogenous origin. In the second place, the failure of Schittenhelm and Wiener[2] to secure a high allantoin output after the feeding of purine material is considered evidence that uric acid is not oxidized to allantoin in the human organism. The findings of Ackroyd to our mind do not constitute a valid objection to our interpretation inasmuch as we were feeding a *uniform diet* and if the food fed did contain allantoin identical quantities of allantoin must of necessity have been ingested daily. And under such conditions the water period witnessed an *increase* in the excretion of allantoin. Much of the force of Schittenhelm and Wiener's findings as furnishing evidence against our interpretation is lost because of the fact that their feeding tests were not adequately controlled and the subjects were not *normal* individuals. On the other hand, an observation which furnishes strong proof of the endogenous origin of allantoin in the human organism is Ascher's finding of allantoin in the urin of a fasting man. More observations on subjects fasting for longer intervals are, however, essential.

[1] Rulon and Hawk, THIS JOURNAL, 32, 1686 (1910).

[2] *Loc. cit.*

When one considers the ease with which uric acid may be oxidized to allantoin *in vitro*, and the further fact that a similar transformation occurs in the organism of lower animals, it is logical to look for at least some oxidation of this character in the human organism.

Summary.

When the diet of a normal man was supplemented by 900 cc. of water per day the average daily output of allantoin (Wiechowski's method) was 0.0135 gram for a period of thirteen days. Upon increasing the water intake to 3450 cc. per day for a period of five days, the average daily allantoin excretion was increased to 0.0173 gram. This constitutes an approximate 20% increase. The daily value for a five-day final period on the original 900 cc. water ingestion was 0.0122 gram.

The increase in the allantoin output accompanying water-drinking may indicate that the oxidative mechanism of the organism has been stimulated through the introduction of the large volume of water into the body and that purine material which would ordinarily have been excreted in some less highly oxidized form has been oxidized to allantoin and excreted in this form. This interpretation is strengthened by the finding in this laboratory of a decreased uric acid output after the water ingestion of the subject (man) had been considerably increased.

In view of the fact that the above interpretation is contrary to the current views regarding purine metabolism in the human organism, the authors make the interpretation tentatively until further experiments may be completed.

URBANA, ILL.

A NEW APPARATUS FOR VACUUM SUBLIMATION.[1]

BY GEORGE W. MOREY.

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An examination of the literature shows surprisingly few descriptions of apparatus for sublimation *in vacuo*. The apparatus of Kempf[2] is probably the best of the common forms. It is easily broken, however, since one of the ground joints becomes heated, both by radiation and by the condensation of the sublimed material. Moreover, it is not suitable for working with large quantities of material.

The need arising to sublime large quantities of certain organic compounds, the apparatus described here was devised. Its construction can be readily seen by referring to the sketch. The bell jar is a large one (26 cm. diameter) and the joint between it and the glass plate is well ground, so that it will readily hold a vacuum with a very thin coating of

[1] Published by permission of the Director of the Bureau of Standards.

[2] Richard Kempf, "Praktische Studien über Vakuum-sublimation," *J. prakt. Chem.*, 78, 201–59.