ON THE PRESENCE OF ALLANTOIN IN CERTAIN FOODS

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Recent investigators who have worked with reliable methods are all agreed that allantoin is a constant constituent of normal human urine when the diet of the subject is of a mixed character. The amount of this allantoin is quite small, not exceeding 15 mgm. a day, and the quantity is not increased when the food contains a large amount of purin substances,[1] or in diseases such as leukaemia.[2, 3] Several observers,[1, 4, 5, 6] have shown also that allantoin, given by the mouth to men, can be recovered to a considerable extent in the urine. It is possible, therefore, that the allantoin found in human urine is not derived from the breakdown of uric acid in the body, but is contained preformed in the food and is excreted unchanged. Schittenhelm and Wiener[1] failed to find allantoin in the urine of a person on a diet composed of milk and vegetables, but Wiechowski[2] found 13-14 mgm. per diem in the urine of two persons on a 'purin-free' diet, and Ascher[3] found its presence in the urine of an individual during starvation. This person, however, starved for 28 hours only, and in the urine which he passed during the last 24 hours only 1 mgm. of allantoin was found; this might have been derived from the food taken on the preceding day, and cannot be accepted as conclusive proof of the endogenous origin of allantoin in man.

It has not been proved that the amount of purin substances present in a food bears any relation to the quantity of allantoin which that food contains. Allantoin has, however, been frequently found in the vegetable kingdom—in the leaf buds of *Platanus orientalis*,[7] of *Acer pseudoplatanus*, of *Acer campestre*, and in the bark of *Aesculus hippocastanum* and of *Acer pseudoplatanus*.[8] It has been found also in the embryos of wheat separated in the process of milling,[9] and in beet juice,[10] but it has not, so far as I know, been looked for in the ordinary foods.

So-called purin-free diet is not generally absolutely purin free, but contains such small traces of purin substances that the uric acid

produced therefrom in metabolism is negligible by comparison with the relatively large amount which has an endogenous origin: thus milk, according to Burian and Schur,[11] contains 0·004—0·006 gram of purin nitrogen per litre, while according to Bessau,[12] it is completely purin free. Most vegetable products also, though not all, contain traces of purin substances, but in most of these the quantity is quite negligible. In the case of allantoin the matter is quite different, for the amount of that substance found in human urine is so small that even traces, if present in a number of foods, may account for all the allantoin excreted.

From this point of view I have looked for allantoin in milk, white bread, eggs, French beans, green peas, bananas and rhubarb, and have found it present in some of these foods in quantities quite sufficient to account for the 13-14 mgm. per diem excreted on a 'purin-free' diet. It was found that milk sometimes contained as much as 0·019 gram per litre, but the quantity is probably very variable; white bread contained 0·006 gram per kilogram, while eggs were completely allantoin free. French beans, which, according to Bessau,[12] contain only 0·0021 gram per cent. of purin nitrogen, were found to contain 0·0287 gram per cent. of allantoin (equivalent to 0·009 gram per cent. of nitrogen), while green peas containing a large quantity of purin nitrogen (0·0274 gram per cent.) contain only 0·00026 gram per cent. of allantoin (equivalent to 0·00009 gram per cent. of nitrogen); bananas and rhubarb were completely allantoin free. Foods, therefore, containing little or no purin substances, may contain relatively large quantities of allantoin, while foods, such as green peas, containing much purin may contain mere traces of allantoin. *The purin content of a food is, in fact, no guide to its allantoin content.* The difficulty of completely extracting and isolating such a substance as allantoin in the pure state from large amounts of foods makes it very probable that the above figures do not represent very closely the amounts actually present, which are almost certainly much greater in the case of bread, French beans, and peas.

If, therefore, allantoin is not destroyed in the human organism, and the amount excreted on a purin-free diet can be accounted for as occurring preformed in the food, and if the amount excreted cannot be increased by the administration of large quantities of uric acid precursors, then the conclusion seems to be justified that uric acid is not converted into allantoin in the course of metabolism in man: but the facts herein referred to leave untouched the much discussed question as to whether man has the power to destroy uric acid in any other way.

Experimental Part

Milk. Three litres of milk, from which the fat had been removed as far as possible by centrifugalisation, were boiled and acidified with acetic acid; the precipitated casein was filtered off, broken up into fine particles, again boiled for a few minutes with two litres of water, and again filtered off on a large 'nutsche.' The two filtrates were added together and completely precipitated by basic lead acetate; the filtrate from this was quite clear, and, after removing excess of lead by sulphuretted hydrogen, and this by a current of air, it was neutralised with sodium hydrate and 20 per cent. solution of mercuric nitrate added till a filtered sample gave a precipitate at once with very dilute allantoin solution; the fluid was then again neutralised and left to stand over night. The precipitate was filtered off through a folded filter, well washed with cold distilled water, suspended in about 300 c.c. of distilled water and decomposed with sulphuretted hydrogen, the mercury sulphide was filtered off and well washed, the filtrate and wash water (500 c.c. in all) were acidified with acetic acid and concentrated to about 250 c.c. on the water bath. This solution was then treated by Wiechowski's method for isolating allantoin, i.e., it was precipitated by phosphotungstic acid in sulphuric acid solution, the excess of phosphotungstic acid and the sulphuric acid removed from the filtrate by agitation with lead oxide till neutral, and again precipitated with basic lead acetate.

After removal of the excess of lead by sulphuretted hydrogen, small samples of the fluid were tested with phosphotungstic acid, with basic lead acetate, and with silver acetate, none of which gave any precipitate. The fluid was then concentrated to about 150 c.c., neutralised exactly with sodium hydrate, and excess of Wiechowski's mercury acetate reagent added; a white flocculent precipitate came down at once; this was left to stand over night. A sample of the clear fluid gave a precipitate at once with a very dilute solution of allantoin. The precipitate was filtered off, suspended in 200 c.c. of distilled water, boiled and decomposed hot with sulphuretted hydrogen; it was then evaporated on the water bath to dryness, the residue extracted with hot water, the mercury sulphide filtered off, and the filtrate evaporated to dryness on the water bath. There was left a deeply pigmented yellowish orange mass; the pigment could not be removed by alcohol, ether, or chloroform, but by careful washing with very small quantities of cold water, and once re-crystallisation from hot water, an almost white

crystalline substance was obtained. This weighed 0·013 gram, had the crystalline form of allantoin, was difficultly soluble in cold water, almost insoluble in alcohol, its aqueous solution was not precipitated by phosphotungstic acid or basic lead acetate. It was not precipitated by silver nitrate alone, but came down at once as a flocculent precipitate on further adding a trace of ammonium hydrate, readily dissolving in excess of the latter, and again coming down on addition of more silver nitrate. It gave the glyoxylic reaction and melted with decomposition at 231° C.; when mixed with an equal quantity of pure allantoin (Merck) the melting point remained the same.

In this experiment the pigment, probably derived from the cow's food, was so difficult to remove, the use of charcoal being inadmissible, that a considerable quantity of allantoin was lost in obtaining it pure.

In a second experiment, three and a half litres of milk were treated in exactly the same way. The final residue was almost colourless; by washing with about $\frac{1}{2}$ c.c. of water and re-crystallising once it was obtained quite white. It had the characters previously described, and melted with decomposition at 232° C.; mixed with an equal quantity of Merck's allantoin the melting point was unchanged. The amount obtained in the pure state was 0·0665 gram, equivalent to 0·019 gram per litre of milk.

Bread. 1100 grams of white bread were cut up into slices and dried in a hot air oven; the dry weight was 740 grams. It was then finely powdered and ground up in a mill to a fine flour. This was boiled with several litres of water; it swelled up into a semi-gelatinous mass, from which no fluid could be removed; it was therefore diluted with water to a thin gruel, and filtered through a large felt jelly bag; the residue was squeezed out as far as possible, and was then again mixed with a large quantity of water and again filtered and squeezed out. The combined filtrates (about 8 litres) were precipitated with basic lead acetate, and the clear filtrate so obtained treated in the same way as described for milk. On evaporating the fluid obtained by decomposition of the mercury acetate precipitate, a gummy residue was left behind, which at first showed no signs of crystallising, but on standing for 24 hours a crystalline substance separated out. This was isolated by washing with a very small quantity of cold water and re-crystallising from a minimal quantity of hot water. 0·0036 gram of a white substance having the crystalline form of allantoin was obtained. Its solutions gave the glyoxylic reaction, and were precipitated by silver nitrate in the presence

of a trace of ammonia, the precipitate being soluble in excess of the latter. It melted with decomposition at 229·5° C.

In a second experiment 2400 grams of white bread were dried and ground up as before; the dried weight was 1618 grams. In order to avoid the swelling up produced by boiling, this was extracted cold for four days with 6 litres of water, toluol being added to prevent bacterial action; it was then filtered through a felt jelly bag, the residue mixed with a further 4 litres of water, and again filtered, the residue being again treated in a similar way. The combined filtrates were precipitated with basic lead acetate, and the clear filtrate obtained treated as above. As in the first experiment, a gummy residue was left, from which crystals slowly separated out; they were isolated in the same manner. 0·0148 gram of a white substance, having all the characters already given for allantoin, was obtained. It melted with decomposition at 231° C., and when mixed with an equal quantity of Merck's allantoin the melting point remained unchanged.

This represents 0·006 gram of allantoin per kilogram of bread, but the difficulty of complete extraction of the bread, and the isolation of the pure substance from the gummy residue obtained at the end, make it very probable that this figure does not represent the whole of the allantoin present in bread.

Eggs. Twelve eggs, weight without shells 510 grams, were well beaten up with three litres of water, acidified with acetic acid, boiled and filtered; the coagulum was broken up into fine particles and re-extracted by boiling with two litres of water, and was again filtered off on a large 'nutsche.' The combined filtrates were concentrated on the water bath to 400 c.c., filtered, and the filtrate precipitated successively by phospho-tungstic acid, basic lead acetate, and silver acetate, exactly as in Wiechowski's method; the filtrate from the silver acetate, after removal of excess of silver by sulphuretted hydrogen, was concentrated on the water bath to 150 c.c., and Wiechowski's mercury acetate reagent added. At first no precipitate came down, but on standing over night a very faint precipitate appeared; the filtrate from this gave a precipitate at once with a dilute solution of allantoin.

The precipitate, when decomposed in the usual way, gave only a slight gummy residue, from which no allantoin could be obtained, nor did it when dissolved in water give the glyoxylic reaction.

Peas. 4500 grams of fresh green peas were finely chopped up in a mincing machine, and extracted for two days with two litres of 98 per

cent. alcohol; this was then filtered off through a jelly bag and the residue squeezed dry in a press; the residue was again extracted for two days with 98 per cent. alcohol, filtered, and pressed as dry as possible. The residue was then extracted twice with petroleum ether to remove fat, and dried in the air; it was then ground up in a mill to a fine flour and extracted with several litres of boiling water, filtered through a jelly bag, and squeezed dry in a press, the residue being again extracted with boiling water, filtered, and pressed dry. The alcoholic extracts were mixed, the alcohol driven off on the water bath, and the remainder added to the two watery extracts. The whole fluid was then precipitated by basic lead acetate, thus obtaining a perfectly clear filtrate, which was afterwards treated in exactly the same manner as described for milk, except that previous to the final precipitation by mercury acetate the fluid was not concentrated below 250 c.c., as it was thought that more allantoin might be present in green vegetables. A gummy residue was obtained from the decomposition of the mercury acetate precipitate, from which, on standing, crystals separated out. By cautiously washing with a small quantity of water these were obtained almost quite white, but were found to consist of two different forms of crystals, one being large columnar crystals, the other prismatic, like allantoin. By fractional crystallisation the allantoin, which was much the less soluble, was obtained pure. The weight isolated was 0·0117 gram; it had all the characters already given for allantoin. The melting point was 230° C. with decomposition, and when mixed with an equal part of Merck's allantoin the melting point remained unchanged.

French beans. 4350 grams of fresh French beans were treated in the same way as that described for peas, the fluid which separated during the mincing being carefully collected and added to the other extracts. The final precipitate with mercury acetate was very voluminous; when it was decomposed and the filtrate evaporated, a comparatively large mass of crystals began to separate out. These were uniform in character, and had the crystalline form of allantoin, but the mother liquor from these crystals gave, an evaporation to dryness, more crystals, some of which resembled allantoin and some did not, being very much larger and columnar. The two substances were separated by fractional crystallisation, allantoin being much the more insoluble.

The total amount of allantoin isolated was 1·2495 grams; this had all the characters already given for allantoin. Its melting point with decomposition was 230° C., which remained unchanged when mixed with

an equal quantity of Merck's allantoin. After one re-crystallisation, 0·1614 gram of the substance was used for nitrogen analysis by Kjeldahl's method, 40·8 c.c. of decinormal acid were required, equivalent to a nitrogen content of 35·39 per cent., the theoretical percentage of nitrogen in allantoin being 35·44 per cent.

Bananas. 445 grams of ripe bananas were mashed up and boiled with one litre of water, and filtered through linen. The filtrate, after precipitation with basic lead acetate, and removal of excess of lead from the filtrate by sulphuretted hydrogen, was evaporated on the water bath to about 200 c.c.; this was then treated by Wiechowski's method. The mercury acetate reagent gave a voluminous precipitate, but no allantoin could be isolated from it, the crystalline substance obtained having none of the characters of allantoin.

Rhubarb. 4350 grams of peeled rhubarb were mashed up and squeezed out in a press, the residue was boiled with water and again squeezed out dry; the filtrate was added to the fluid already obtained, and the whole precipitated by basic lead acetate. The filtrate, after removal of excess of lead by sulphuretted hydrogen, was concentrated in vacuo to about 400 c.c.; it was then treated by Wiechowski's method. Only a very faint precipitate came down with the mercury acetate reagent, from which no allantoin could be isolated.

Conclusions

The whole quantity of allantoin excreted by man on a milk and vegetable diet may be derived directly from the food. Milk, white bread, French beans, green peas, all contain small quantities of allantoin, while none could be isolated from eggs, bananas, or rhubarb.

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