

TOXICITY OF PHENYLACETIC ACID.

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(Received for publication, October 7, 1919.)

The retention of protein material in the intestine and its subsequent putrefaction leads to the formation and absorption of many protein decomposition products which are more or less harmful to the organism.

Phenylalanine on putrefaction yields three different aromatic acids, phenylpropionic acid, phenylacetic acid, and benzoic acid, while tyrosine undergoes analogous decomposition.

However, if phenylpropionic acid (1) is introduced into the gastrointestinal tract and absorbed, no phenylacetic acid is formed but it is subjected to the process of β -oxidation, loses two C atoms, and is changed directly into benzoic acid.

The benzoic acid combines with glycocholl and is excreted in the urine as hippuric acid. *p*-Hydroxyphenyl propionic acid (1) in a like manner is oxidized to *p*-hydroxybenzoic acid and is excreted as *p*-hydroxyhippuric acid. Phenylacetic acid resists oxidation and remains to be altered by combination with other compounds.

Salkowski (2) considered the acid relatively non-toxic and believed that it existed even in normal human urine as the free acid. This was perhaps because he had found only the combined acid in the urine of animals, such as dogs (2, 3), rabbits, and horses and was unable to isolate either the free or combined acid from human urine.

Huppert (4) fed phenylacetic acid to patients suffering from alkaptonuria and proved that the acid aided no way in the formation of homogentisic acid but was unable to find even a trace of the acid after feeding the patient a 10 gm. dose.

Hotter (5), who ingested the acid himself, could find neither the combined or uncombined acid in his urine, so concluded

that it was oxidized to benzoic acid and excreted as hippuric acid. Phenylacetic acid is particularly interesting from a physiological standpoint on account of its different metabolic action in the organism of man, animal, and fowl.

In the human body, the acid is combined with glutamine and excreted as phenylacetyl glutamine (6). This is the only case so far recorded where the amino-acid glutamine has been used by the body for the purpose of detoxicating a poisonous substance. Animals fed on phenylacetic acid detoxicate it by joining it with glycocoll and excreting it as phenaceturic acid (2).

After feeding the acid to a hen, Totani (7) isolated a compound from the excreta which he terms phenacetornithuric acid. This substance is a combination of one molecule of ornithine with two molecules of phenylacetic acid.

Phenylacetic acid, while found in only small amounts in the normal human body is one of the most important protein putrefaction products and is by no means as non-toxic as was previously supposed.

A hen weighing 2.23 kilos, after receiving 1 gm. of the acid, refused to eat. A second dose of 1 gm. 3 days later caused the hen to lose weight and develop marked signs of intoxication. A dog weighing 32.6 kilos was able to take 3 gm. of the acid with no apparent signs of discomfort; however, after receiving a dose of 7 gm. of the acid it became very thirsty, refused to eat, seemed to be greatly nauseated, and vomited several times.

A monkey of 4.2 kilos body weight, which received a dose of 1 gm. and refused to eat for several days, also developed a marked diarrhea. Twelve adult humans (male) after ingesting 5 gm. each of the acid showed in every case practically the same symptoms. The sodium salt of the acid was dissolved in 200 to 300 cc. of water and rapidly drunk. In 15 to 30 minutes after the ingestion of the acid, the subject became thirsty and this symptom was rapidly followed by a feeling of hunger. If food was ingested, symptoms of nausea developed; in case no food was taken, a feeling of dizziness resulted, followed either by drowsiness or increased nervousness. One subject weighing 59.1 kilos ingested as much as 16 gm. of the acid within a period of 2 hours. Within a few minutes, the usual sensation of dizziness and hunger developed, so the subject partook of an unusually heavy meal and went to bed.

1 hour after ingesting the last of the acid, he was unable even to stand unsupported. After sleeping soundly for 6 hours, he awoke and drank 1 liter of water, immediately fell asleep, and again slept soundly for nearly 8 hours. On waking he demanded water and drank more than $1\frac{1}{2}$ liters. He complained of nausea, headache, pain in the eyes, and of loud ringing in the ears. He was able to sit alone but seemed unable to stand unsupported or to correlate his movements. After another 4 hours of sleep, he appeared quite refreshed and normal in every way. In this case there was no sign of diarrhea but on the contrary he presented an obstinate case of constipation, which lasted for about 3 weeks.

In many respects the symptoms of poisoning by this acid resemble those of alcoholic poisoning.

EXPERIMENTAL.

In order to determine the toxicity of the acid, we decided to feed a small dog increasing doses of the substance and to determine if possible the minimum dose which would cause death and to study as carefully as possible any pathological changes produced by the acid.

A small dog of 7.5 kilos body weight was selected and placed in a metabolism cage for observation. The acid was fed to the dog as a water solution of the sodium salt by means of a stomach tube. On the 1st day of the experiment, he received 1 gm. of the acid. On each succeeding day, the dose of the acid was increased by 1 gm.

During the first 24 hours of the experiment following the 1 gm. dose of the acid, the dog showed no signs of discomfort but ate as usual and showed no signs of abnormal thirst. On the 2nd day after receiving the 2 gm. of the acid, the dog showed an abnormal appetite and drank often but only a small amount of water each time. On the 3rd day, he showed signs of drowsiness, but ate as much as usual and drank a large amount of water. On the 4th day, the dog refused to eat, spent most of the time in sleep, and seemed scarcely able to stand when removed from the cage. He was unable to walk and weighed at this time only 6.35 kilos. On the 6th day he still refused to eat and seemed to be in a semicomatose condition.

Up to this time there had been no signs of albumin in the urine but at this point a sufficient quantity was present to give a decided reaction. On the 7th day of the experiment, the dog appeared very weak and after receiving 7 gm. of the acid, underwent a series of convulsions during which time he vomited most of the acid. As much of this acid as possible was reclaimed and weighed. The total amount vomited was approximately 5.5 gm. so the dog received in fact only about 1.5 gm. of the phenylacetic acid on the 7th day.

For about $2\frac{1}{2}$ hours after receiving this last dose of the acid, he appeared quite lifeless, then suddenly underwent a second series of convulsions, which ended in death.

Autopsy.—Performed about 6 hours after death. Male dog weighing 6.30 kilos. Gross examination of the organs presented no morphological lesions with the exception that the kidneys on section were congested and somewhat swollen, the cortex being pale; medulla congested, capsule non-adherent. Portions of liver, kidney, spleen, stomach, and alimentary canal were taken for microscopic examination. The specimens were fixed in Orth's fluid and mounted in paraffin. Sections were cut 6 microns in thickness and examined with $\frac{1}{2}$ oil immersion, ocular 10.

Microscopic Examination.—The tunica fibrosa of the kidney does not appear to be thickened and the nuclear elements show no deficiency in staining qualities. The capillaries in the cortex corticis are engorged and the cellular elements within them appear disintegrated. While there is a general engorgement of the blood vessels, of the cortical portion of the organ, there is no extravasation of blood in the interstitial tissue.

The epithelium of the proximal convoluted tubules is much swollen and granular, so that the lumen of the tubule is in many places completely occluded by the approximation of the distal edge of the epithelial cells. Some of the tubules contain in their lumen the remains of broken down epithelial cells but this is in localities distinct from those in which the lumen is occluded and may indicate epithelial areas, which bore the effects of a greater toxic action of the drug. Blood elements are not seen within the tubules.

A glistening, hyaline material is found in the lumen of many of the tubules and in the cytoplasm of many cells the same material is seen. The engorgement of the capillaries between the tubules is marked. The degeneration of the epithelium is most marked in the proximal convoluted tubules in the neighborhood of the Malpighian corpuscles, becoming less marked as the descending loop is approached.

The arched collecting tubules are filled with the hyaline material and their epithelium, including the nuclei, is in many places destroyed. The epithelium of Bowman's capsule is likewise destroyed in many of the

renal corpuscles and while shrinkage of the glomerulus from the capsule is not present in every instance, yet it occurs in many of the corpuscles, particularly in those near the boundary zone of the medulla, and in the capsular space an exudate of hyaline and granular material and blood cells is present.

A round cell infiltration of the stroma of the glomerulus occurs and the capillaries of the tuft are engorged with blood.

In the medulla of the kidney, both limbs of Henle's loop show marked destructive changes of their epithelium. This in many places is totally disintegrated, so that the lumen of the tubules is filled with a mass of cellular remains and misplaced nuclei and a fine reticular mass, staining deeply with eosin, is present. Such epithelial cells as are not destroyed are detached in places from the wall of the tubules.

The epithelium of the straight collecting tubules does not appear affected by the action of the drug. All the cells are in place, the nuclei distinct, and the cytoplasm is clear. The nuclear membrane is distinct and the lumen of the tubule, for the most part, empty.

The interstitial tissue was not altered in appearance or amount.

The microscopic examination of the liver shows the cells to contain a number of globular refracted spaces, varying in size and in some instances occupying the greater part of the cell. The staining quality of many of the nuclei of the liver cells was markedly deficient. Unfortunately a section was not stained for fat.

The spleen was negative.

The stomach and intestines presented nothing of note.

SUMMARY.

The microscopic findings would seem to indicate that as a result of excessive doses of phenylacetic acid in the dog, the secreting epithelium of the proximal convoluted tube of the kidney is markedly affected; that the endothelium of the blood vessels is not affected; that the epithelium of the arched collecting tubule shows evidence of a destructive action, while that of the straight collecting tubule appears to escape.

The secreting epithelium of the limbs of Henle's loop is most distinctly involved; the fact that the interstitial tissue of the kidney is not injured and that the liver changes is in all probability secondary.

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