

## COMPARATIVE METABOLISM OF CERTAIN AROMATIC ACIDS.

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### *Fate of Phenylacetic Acid in the Organism of the Monkey.*

Many experiments have been reported showing a marked difference between metabolism in the human body and in the lower animals. This alone has stimulated considerable interest on account of the apparent lack of reason for such difference. In the last few years experiments have been carried out with animals more closely related to the human in order to compare their metabolism with that of both human beings and lower animals.

Hunter and Givens (1) report the purine metabolism in the monkey as resembling that of the lower animals and not the human. In the urine of the monkey (*Cercopithecus*) allantoin accounted for 73 per cent of the nitrogen arising from the catabolism of the endogenous purines while the remainder appeared principally as purine bases and none as uric acid. Later work by the same authors (2) shows like results for another monkey (*Cercopithecus callitrichus*). Wiechowski (3) obtained results comparable to those of Hunter and Givens but was unable to find allantoin as the end-product of the purine metabolism of the chimpanzee. Baumann and Oviatt (4) found the urinary sulfur excretion in the monkey (*Macacus*) quite different from that of man. In the case of the monkey the ratio of inorganic to ethereal sulfates was approximately  $3\frac{1}{2}$  to 1 while for man it bore the ratio 12 to 1.

An interesting phase of this same question is the manner in which the different animal organisms detoxicate certain protein putrefactive products. The *series of compounds* resulting from the action of putrefactive bacteria on the aromatic amino-acids must be detoxicated and eliminated from the animal body as

soon as possible. This detoxication process seems often to differ for different species or at least results in the excretion of an entirely different end-product for the acid thus resorbed.

Phenylacetic acid is one of those aromatic acids which yield different conjugation compounds in the urine after ingestion by men and animals respectively. Phenylacetic acid in combination with glycocoll is excreted normally by many of the herbivora as phenaceturic acid. Phenaceturic acid was discovered by Salkowski (5) in normal horse urine. Phenaceturic acid has been found in the urine of many different animals after feeding phenylacetic acid. Thus E. and H. Salkowski (6) isolated it from the urine of dogs and rabbits, and Vasiliu (7) from the urine of sheep after phenylacetic acid feeding. E. Salkowski (8) believed he had found traces of the phenaceturic acid in normal human urine. Totani (9) isolated from the excreta of chickens phenylacetylornithuric acid after phenylacetic acid feeding. Thierfelder and Sherwin (10) found in human urine two compounds, phenylacetyl glutamine and phenylacetyl glutamine urea after various amounts of phenylacetic acid had been ingested but were unable to find even a trace of either phenaceturic acid or uncombined phenylacetic acid.

Before beginning certain metabolism work on a monkey it was important to know whether the fate of the phenylacetic acid in the organism of the monkey would resemble that of the human organism or that of the lower animals. The combination with glycocoll resulted showing the metabolic process to resemble that of the lower animals, not only in the case of the phenylacetic but also for the *p*-hydroxyphenylacetic and *p*-hydroxy benzoic acid (11)

#### EXPERIMENTAL.

A 4.2 kg. female monkey (*Macacus rhesus*) was placed on a regular diet of milk, bread, bananas, and apples for several days in order to see if the urine contained traces of any phenylacetic acid compound. After no compound of this nature could be found in the urine, the monkey was fed 1 gm. of phenylacetic acid per day as the soluble sodium salt. The sodium salt dissolved in fresh milk at first seemed quite acceptable, but after the 3rd day it was refused. Any food or liquid possessing even the slightest odor of phenylacetic acid was entirely refused by the monkey. At this point forced feeding was resorted to and the water solution of the sodium salt introduced directly into the stomach by means of a stomach tube. The

amount thus given varied between 1 and 2 gm. The physiological effect seemed to be much the same as that produced on human beings. Each dose was followed by a period of dullness and inactivity, while the ingestion of 2 gm. (0.47 gm. per kg. body weight) resulted in an entire loss of appetite for 24 hours. When the phenylacetic acid is ingested by men in quantities varying from 0.05 to 0.26 gm. per kg. body weight intoxication results much the same as that produced by alcohol.

The monkey's urine was collected for 36 hours after each dose of the acid, and the different portions were united and acidified with phosphoric acid until a distinct acid test with Congo red resulted. The concentrated urine was then extracted repeatedly in a liquid extracting apparatus with ethylacetate until no phenylacetic acid compound formed by evaporation of the last extraction. The ethylacetate extract thus prepared was evaporated to one-half its original volume and placed on ice for 24 hours. As no phenylacetyl glutamine crystallized out at this concentration, the extract was again evaporated and the evaporation continued on subsequent days until the entire ethylacetate solution amounted to about 100 cc. only. At this concentration, there being no possibility of obtaining any phenylacetyl glutamine, the extract was evaporated carefully almost to dryness and then taken up with 50 cc. of water and boiled with charcoal to remove the pigment. After filtering this water solution from the charcoal, evaporating, and allowing the concentrated solution to stand on ice, there appeared small rhombic crystals of phenaceturic acid. The mother liquor from these crystals was optically inactive, showing again the absence of phenylacetyl glutamine. After the feeding of 1 gm. of phenylacetic acid the amount of phenaceturic acid isolated from the urine amounted to only 0.82 gm. or 51 per cent of the expected yield.

The phenaceturic acid was twice recrystallized from water, dried *in vacuo*, and then used for analyses.

Melting point 142-143°.

0.1232 gm. of the acid required 6.29 cc. of 0.1 N sodium hydroxide instead of 6.37 cc., the theoretical amount.

0.1331 gm. substance gave 8.50 cc. nitrogen at 21° and 748 mm. pressure.

	Calculated for C <sub>10</sub> H <sub>11</sub> NO <sub>2</sub> :	Found:
N.....	7.25	7.15

#### CONCLUSION.

The metabolism of phenylacetic acid in the organism of the monkey is the same as that found in the lower animals and entirely different from the metabolism of the same substance in man.

Phenylacetic acid in the monkey is conjugated with glyco-coll and excreted as phenaceturic acid, while in man it is conjugated with glutamine and excreted partly as phenylacetyl glutamine and partly as phenylacetyl glutamine urea.

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