ARTICLE:

THE MAXIMUM PRODUCTION OF GLUTAMINE BY THE HUMAN BODY AS MEASURED BY THE OUTPUT OF PHENYLACETYLGLUTAMINE

Carl P. Sherwin, Max Wolf and William Wolf

J. Biol. Chem. 1919, 37:113-119.

THE MAXIMUM PRODUCTION OF GLUTAMINE BY THE HUMAN BODY AS MEASURED BY THE OUTPUT OF PHENYLACETYLGLUTAMINE.

By CARL P. SHERWIN, MAX WOLF, and WILLIAM WOLF.

(*From the Laboratory of Fordham University Medical School, New York.*)

(Received for publication, November 12, 1918.)

Phenylacetic acid when fed to animals such as horses (1), dogs, rabbits (2), and sheep (3), is excreted in the urine as phenaceturic acid. This combination of phenylacetic acid with glycocoll, however, does not take place in the human body. In man the phenylacetic acid combines with glutamine and is excreted in the urine as phenylacetylglutamine (4). E. Salkowski and H. Salkowski (2) find after feeding phenylacetic acid to rabbits an increase of almost 75 per cent in the total SO_3 output, but at the same time a decrease in the amount of "combined SO_3." The increase in the amount of inorganic sulfates in the urine they attribute to an increased catabolism of protein material necessary for the preparation of sufficient amount of glycocoll to detoxicate the phenylacetic acid. The decrease in ethereal sulfates they believed to be due to the action of phenylacetic acid as a disinfecting agent with a subsequent decrease in the amount of intestinal putrefaction.

As glutamine (5) rather than glutamic acid seems to be the primary amino-acid constituent of protein, we thought it important to determine the maximum amount of this acid which the human body is able to furnish for the detoxication of phenylacetic acid. We wished also to know whether the ingestion of phenylacetic acid would be followed by increased sulfur metabolism in the human body as reported by previous investigators (2) in animals. Lastly, we wished to determine the effect of the acid in the course of intestinal putrefaction as measured by the excretion of urinary indican, as well as by the amount of ethereal sulfates excreted in the urine.

EXPERIMENTAL.

The subject was a man weighing 60.2 kilos. He was maintained throughout the entire experimental period on a uniform diet. The experiment covered a period of 24 days. On the 5th day of this period 2.5 gm. of phenylacetic acid were ingested, on the 7th day 5 gm., on the 10th day 7.5 gm., on the 13th day 10 gm., and on the 21st day 15 gm. The acid in each case was ingested as a solution of the sodium salt. The entire amount of the solution was taken at once, covering a period of not more than 3 to 5 minutes. The urine was collected in 24 hour periods, beginning at the time of the ingestion of the acid. The urine volume was first measured, and sufficient amount taken for the indican and sulfate determinations on that day and the day following the acid ingestion; the remaining urine volume was measured, exactly neutralized with sodium carbonate, and evaporated on the water bath to a thick syrup. The determination of the indican was made in duplicate upon each urine sample by the method of Ellinger (6). Each cc. of the Wang solution (7) employed in the titration was found upon standardization to be equivalent to 0.300 mg. of indigo,[1] or 0.576 mg. of indican. The ethereal sulfates and total sulfates in the urine were determined by the method of Folin (8). To determine the quantity of phenylacetylglutamine, the evaporated urine was made acid with phosphoric acid (Congo red as indicator) and extracted in a continuous extracting apparatus with absolute ethyl acetate. The amount of ethyl acetate used during each extraction was about 300 cc., and the time of extraction varied between 1 and 2 hours. The extracting was continued until the ethyl acetate was found on evaporation to contain no more phenylacetylglutamine. To obtain the phenylacetylglutamine the ethyl acetate extracts were placed on ice for 24 hours. At the end of this time, the white flaky crystals of the substance appeared on the sides and bottom of the flask. These crystals were scraped loose and filtered off, the mother liquor was evaporated to one-half the volume, and again placed in the ice box for 24 hours. This process was repeated until all the compound was crystallized out of the solution. The

[1] The indigo used in standardizing the Wang solution was furnished by Professor P. B. Hawk of Jefferson Medical College.

phenylacetylglutamine from the different extractions was united and recrystallized from absolute ethyl acetate, dried, and weighed. In each extract there occurred about an equal amount of phenylacetylglutamine and phenylacetylglutamine urea. In order to split off the urea and convert the urea compound into phenylacetylglutamine, a water solution of the urea compound was made slightly alkaline with barium hydroxide, the solution treated with carbon dioxide to remove the excess of barium, filtered, and the filtrate evaporated to dryness at a low temperature on the water bath. The residue, consisting of the barium salt of phenylace tylglutamine and urea, was extracted several times with hot absolute alcohol to remove the urea. The insoluble barium salt was separated from the urea by filtration. The barium salt of the phenylacetylglutamine was dissolved in a small amount of water, made acid with phosphoric acid, the barium sulfate filtered off, and the phenylacetylglutamine extracted from the concentrated solution with ethyl acetate.

DISCUSSION.

The results of the experiment are summed up in Table I.

On account of the toxicity of the phenylacetic acid, it was impossible for the subject to ingest more than 15 gm. of the acid. Even 5 gm. caused thirst, a slight feeling of dizziness, and nausea. The 10 gm. dose seemed to produce no exaggeration of these symptoms, but after ingestion of 15 gm. the subject noticed marked signs of poisoning, not unlike those following the ingestion of large quantities of alcohol. The daily urine volume which generally measured 800 to 900 cc. rose to at least 1,000 cc., and in one instance to 1,300 cc. after the ingestion of more than 5 gm. of phenylacetic acid. The increase in sulfur metabolism noted by Salkowski and Salkowski (2) after feeding the same acid to rabbits is not borne out in this experiment.[2] On the 13th day of the experiment after an ingestion of 10 gm. of the acid, there was a rise in the total SO_4 output from 1.6862 gm. on the previous day to 2.5107 gm. This, although a marked rise, is even lower than the amount excreted the 3rd day before the ingestion of any of the acid.

[2] The effect of phenylacetylglutamine production on nitrogen metabolism is being studied and will be reported later.

TABLE I.

Day of experiment.	24 hr. urine, volume.	Phenylacetic acid ingested.	Phenylacetylglutamine recovered from urine.	Phenylacetic acid conjugated with glutamine.	Phenylacetic acid recovered from urine as phenylacetylglutamine.	Glutamine extracted from urine as phenylacetylglutamine.	Total SO_4 excreted in urine during 24 hrs.	Inorganic SO_4 excreted in urine during 24 hrs.	Ethereal SO_4 excreted in urine 24 hrs.	Indican excreted in urine during 24 hrs.	Relation of ethereal SO_4 to total SO_4 for 24 hr. period.	Relation of indican SO_4 to ethereal SO_4.
	cc.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*		
1	860						1.8049	1.5905	214.4	23.82	1: 8.41	1: 19.96
2	850						1.8467	1.6843	162.4	27.26	1: 11.37	1: 13.21
3	960						2.6013	2.3340	267.3	26.44	1: 9.73	1: 22.42
4	920						2.5340	2.3518	182.2	22.86	1: 13.41	1: 17.67
5	900	2.5	2.02	1.1332	52.88	1.1070	2.1040	1.9683	135.7	12.90	1: 15.48	1: 23.33
6	1,010						2.7792	2.5702	209.0	28.16	1: 13.31	1: 16.46
7	1,100	5.0	6.16	3.3820	67.67	3.3264	2.7491	2.5848	164.3	00.00	1: 16.73	1: 00.00
8	970						1.9050	1.6972	207.8	00.00	1: 9.16	1: 00.00
9	820						2.3440	2.1473	196.7	12.66	1: 11.91	1: 34.44
10	1,070	7.5	7.23	3.7241	49.65	3.6873	2.4944	2.3516	132.8	14.28	1: 14.86	1: 20.63
11	820						2.0066	1.9471	159.5	9.76	1: 12.54	1: 36.24
12	785						1.6862	1.5314	154.8	5.39	1: 10.89	1: 63.64
13	950	10.0	10.07	5.1870	51.87	5.1357	2.5107	2.3237	167.0	31.89	1: 15.03	1: 12.00
14	840						1.6403	1.4835	156.8	27.32	1: 10.46	1: 12.76
15	700						1.8446	1.6903	154.3	28.11	1: 11.95	1: 12.18
16	900						1.7648	1.6272	137.6	29.02	1: 12.82	1: 10.53
17	1,200						1.6680	1.4850	183.0	30.24	1: 9.11	1: 13.42
18	690						1.5998	1.4688	131.0	19.57	1: 12.21	1: 14.84
19	815						1.7077	1.5573	150.4	22.61	1: 11.35	1: 14.75
20	820						2.3319	2.0873	244.6	26.59	1: 9.53	1: 20.40
21	1,305	15.0	14.75	7.5977	50.65	7.5225	2.0398	1.9034	105.5	22.39	1: 19.31	1: 10.46
22	860						2.2621	2.0654	196.7	40.87	1: 11.49	1: 10.65
23	780						1.9707	1.8060	164.9	6.27	1: 11.95	1: 58.30
24	720						1.8294	1.6830	146.4	8.70	1: 12.50	1: 37.32

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.