[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY OF FORDHAM UNIVERSITY]

SYNTHESIS OF AMINO ACIDS IN ANIMAL ORGANISMS. I. SYNTHESIS OF GLYCOCOLL AND GLUTAMINE IN THE HUMAN ORGANISM

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The fact that the human body is able to furnish glycocoll for the conjugation with benzoic acid has long been known and has been studied by many investigators.

Lewinski[1] observed neither benzoic acid nor benzoyl glycuronates in the urine of men to whom 12 to 20 g. of benzoic acid had been fed in small doses during the course of 12 hours. After feeding doses of 25 to 40 g., small amounts of benzoic acid (about 25% of the intake) were excreted as the free acid along with certain dextro-rotatory substances of a reducing nature indicating the presence of glycuronates. One may conclude from this that the average human adult is able to convert quantitatively about 20 g. of benzoic acid into hippuric acid. Brugsch[2] and Tsuschija,[3] on the contrary, were able to recover only about 25 to 50% of the ingested benzoic acid as hippuric acid. Dakin[4] found in his work with benzoic acid that doses of 5 to 10 g. of the acid ingested per day were almost entirely converted into hippuric acid before its elimination in the urine. The human body like that of the lower animals is therefore able to synthesize glycocoll within reasonable limits under conditions where no glycocoll occurs in the protein of the diet or even in a protein-free diet. Wiechowski[5] maintained that the glycocoll content of the tissues is insufficient to account for the quantity of glycocoll thus eliminated and that glycocoll can be synthesized at the expense of urea formed under normal conditions. Ringer[6] observed in his work an increase in the nitrogen elimination after feeding benzoic acid to animals and suggested that glycocoll resulted from the "extra destroyed protein" during this period. Epstein and Bookman,[7] experimenting on rabbits, concluded that benzoic acid as a toxic substance acts in a selective way causing the elimination of excessive amounts of nitrogen which is mostly excreted as hippuric acid nitrogen. McCollum and Hoagland[8] brought a pig of 46.7 kg. body weight into a condition of minimal nitrogen metabolism by feeding a diet of starch containing 75 calories for each kilogram of body weight. The diet was continued and increasing amounts of benzoic acid were fed. The results of their experiment show that when protein metabolism is reduced to a minimal level by carbohydrate ingestion the addition of benzoic acid does not affect the creatinine output, affects but little the total nitrogen, but may reduce the total elimination of urea nitrogen from 56% of the total nitrogen output to 19% of the total. This difference of 37% of the total nitrogen which is ordinarily converted into urea is under these circumstances eliminated as glycocoll.

Lewis[9] kept a man on a low protein diet containing no glycocoll for a period of 3

[1] Lewinski, *Arch. exp. Path. Pharm.*, **58,** 397 (1908).

[2] Brugsch, *Z. exptl. Path. Therap.*, **5,** 731 (1909).

[3] Tsuschija, *ibid.*, **5,** 737 (1909).

[4] Dakin, *J. Biol. Chem.*, **8,** 103 (1909).

[5] Wiechowski, *Beitr. chem. Physiol.*, **7,** 204 (1906).

[6] Ringer, *J. Biol. Chem.*, **10,** 327 (1911).

[7] Epstein and Bookman, *ibid.*, **10,** 353 (1911).

[8] McCollum and Hoagland, *ibid.*, **16,** 299 (1913–1914).

[9] Lewis, *ibid.*, **18,** 225 (1914).

days. On the second day the subject ingested 8.47 g. of benzoic acid and the urine was collected at 2-hour intervals. Urea nitrogen and ammonia nitrogen were determined as one. During the first 2-hour period the amount of this nitrogen dropped from 80% to 67% of the total nitrogen; during the second 2-hour period, to 61.6%, and during the third to 64.9%, after which it returned to normal. This shows that the ingestion of 8.47 g. of benzoic acid may increase slightly the output of total nitrogen in the urine but at the same time cause a decided decrease in the amount of urea nitrogen plus ammonia nitrogen eliminated.

Thierfelder and Sherwin[10] have shown that glutamine is furnished by the human body for the detoxication of phenyl acetic acid and it was later found by Sherwin, M. Wolf and W. Wolf,[11] that 7.5 g. of glutamine was synthesized by the body on glutamine-(glutamic acid) free diet for the detoxication of ingested phenylacetic acid without materially increasing protein metabolism as shown by the total nitrogen and sulfur elimination.

In previous work a human subject had been reduced to a low protein diet[9] for a period of only 3 days and benzoic acid fed on only 1 day. It seemed to us important that the subject be reduced to an endogenous level of nitrogen metabolism, if possible, and the experiment be extended over a longer period. We also decided to feed first benzoic acid, then phenylacetic acid and lastly a mixture of the two in order to answer if possible the following questions: (1) What is the maximum amount of urea nitrogen which the human body will "side track" for the synthesis of glycocoll, and what influence if any has this on the other nitrogen constituents of the urine? (2) Can glutamine be synthesized at the expense of the urea nitrogen alone or does the amino and amide nitrogen necessary for this compound come from different sources? (3) Can glycocoll and glutamine be formed simultaneously in the human body, and if so which one is more readily formed when a limited supply of urea nitrogen is available?

The subject, a healthy man of 80 kg. body weight, was maintained on a diet of cream, bananas, and starch or lactose for a period of 7 days.

TABLE I

INGESTED FOOD PER DAY, AND ITS ENERGY VALUE

Period	Starch	Lactose	Bananas	Cream	Total energy	Nitrogen ingested in food
Days elapsed	G.	G.	G.	G.	Calories	G.
1	180	0	50	435	2475	0.080
2	50	120	325	100	1186	0.520
3	100	120	380	30	1314	0.610
4	100	110	420	10	1278	0.670
5	40	200	545	5	1581	0.870
6	40	200	335	0	1276	0.535
7	40	150	450	0	1197	0.720

[10] Thierfelder and Sherwin, *Ber.*, **47**, 2630 (1914); *Z. physiol. Chem.*, **94**, 1 (1915).

[11] Carl P. Sherwin, M. Wolf and W. Wolf, *J. Biol. Chem.*, **37**, 113 (1918).

TABLE II—NITROGEN DATA

Period	Phenylacetic fed	Benzoic fed	Length of period	Urine Volume	Nitrogen: Total	Nitrogen: Urea	Nitrogen: Ammonia	Nitrogen: Creatinine	Nitrogen: Glycocoll	Nitrogen: Glutamine	Percentage of total nitrogen: Urea	Percentage of total nitrogen: Ammonia	Percentage of total nitrogen: Creatinine	Percentage of total nitrogen: Glycocoll	Percentage of total nitrogen: Glutamine	Percentage of total nitrogen: Rest of Nitrogen	Amt. acid detoxicated: Benzoic	Amt. acid detoxicated: Benzoic fed	Amt. acid detoxicated: Phenylacetic	Amt. acid detoxicated: Phenylacetic fed
	G.	G.	Hrs.	Cc.	G.	G.	G.	G.	G.	G.	%	%	%	%	%	%	G.	%	G.	%
1	...	...	24	2100	15.88	12.3	1.15	0.62	....	..	77	7	4	...	..	12	...	...	...	..
2	...	...	24	2020	5.91	4.66	0.52	0.58	....	..	77	9	10	...	..	4	...	...	...	..
3	...	...	24	1000	5.71	4.23	0.45	0.56	....	..	74	8	10	...	..	8	...	...	...	..
4	...	...	8	785	2.40	1.86	0.14	0.21	....	..	77	6	8	...	..	9	...	...	...	..
	...	3.0	6	685	1.95	0.93	0.18	0.15	0.32	..	48	9	8	17	..	18	2.8	93	...	..
	...	3.0	6	250	1.20	0.64	0.09	0.12	0.31	..	52	7	9	26	..	5	2.7	90	...	..
	...	...	4	53	0.54	0.35	0.05	0.05	..	..	65	10	10	...	..	15	...	...	...	..
Totals............			24	1773	6.09	3.78	0.46	0.53	0.63		60	8	9	10		13	5.5	92		
5	3.3	...	6	705	1.54	0.78	0.11	0.16	....	0.30	50	7	11	...	20	12	...	...	1.5	44
	...	...	6	415	1.77	0.69	0.14	0.15	....	0.35	39	8	9	...	20	24	...	...	1.7	51
	3.3	...	6	250	1.18	0.81	0.08	0.15	....	0.11	68	7	12	...	9	4	...	...	0.5	16
	...	...	6	135	0.96	0.34	0.10	0.15	....	0.30	35	10	16	...	31	8	...	...	1.4	44
Totals............			24	1505	5.45	2.62	0.43	0.61	0.31	1.06	48	8	11	...	19	14	...	...	5.1	77
6	3.3	3.0	3	820	1.07	0.20	0.09	0.10	....	0.31	19	8	10	29	29	5	2.7	89	1.5	46
	...	...	3	286	0.83	0.38	0.06	0.12	....	0.20	45	8	14	...	24	9	...	...	1.0	30
	...	...	3	222	0.66	0.46	0.05	0.11	....	0.02	70	8	17	...	3	3	...	...	0.1	3
	...	...	15	675	2.22	1.68	0.21	0.27	....	....	76	9	12	...	..	3	...	...	...	..
Totals............			24	2003	4.78	2.72	0.41	0.60	0.31	0.53	57	8	13	6	11	5	2.7	89	2.6	79
7	4.0	...	4	750	1.00	0.44	0.06	0.12	....	0.32	44	6	12	...	30	8	...	...	1.5	39
	...	...	4	555	1.31	0.32	0.14	0.11	....	0.50	24	11	8	...	38	18	...	...	2.4	60
	6.0	...	4	520	1.01	0.29	0.10	0.10	....	0.38	29	10	10	...	37	14	...	...	1.8	30
	...	...	4	255	0.97	0.12	0.10	0.09	....	0.51	12	10	9	...	52	16	...	...	2.5	41
	...	...	8	495	1.26	0.40	0.20	0.22	....	0.26	32	16	18	...	21	14	...	...	1.3	21
Totals............			24	2575	5.55	1.57	0.60	0.64		1.97	28	11	12		21	14			9.5	95

The preceding table shows the weight of each substance eaten per day as well as its calorific value.

This diet which is practically nitrogen free insofar as utilizable nitrogen is concerned[12] is also of sufficient calorific value for the needs of a man undergoing only a small amount of physical exertion. Agar-agar was ingested in sufficient amounts to cause the regular evacuation of the intestine and thus avoid the absorption of nitrogenous end products of putrefaction.

In Table II, the days of acid feeding have been divided into sub-periods of varying length in order to study the amount of different substances excreted immediately after the ingestion of a given amount of acid. The acid ingested during a certain sub-period of the day was always taken at the beginning of that sub-period, and the entire output of urine collected during each sub-period was treated as a single unit. Also for each one of the last 4 days a sum total is given of all the sub-periods that the entire 24-hour period may be compared to one of the first 3 days of the control period.

A brief survey of Table II brings out clearly several interesting facts. Neither benzoic acid nor phenylacetic acid when fed in moderate doses (3 to 10 g.) to man acts as a stimulator of endogenous metabolism when the subject has been reduced practically to a state of endogenous protein catabolism. This is shown by the fact that the amount of total nitrogen excreted is not increased during the period of acid ingestion. It is also seen that neither glycocoll nor glutamine is built at the expense of "extra destroyed protein" but rather at the expense of one or more of the nitrogenous constituents of the urine, and of these only urea is affected to a marked degree.

Urea, during the control period maintained an average of 74 to 77% of the total nitrogen, while during the feeding period it dropped to 60%, 57%, then to 48% and finally to 28% of the total nitrogen of the entire 24-hour period. If taken by sub-periods even more striking evidence is found of the inroads made on urea nitrogen for the purpose of glycocoll or glutamine synthesis. After a dose of 3 g. of benzoic acid on the fourth day, followed at the end of 6 hours by a second dose of 3 g. the urea nitrogen dropped to 48% and 52% respectively of the total nitrogen. On the fifth day 2 doses each of 3.3 g. of phenylacetic acid caused a further drop of the urea nitrogen to 39% and 35% respectively of the total nitrogen. It is interesting here to note that the effect of the benzoic acid on the urea nitrogen was immediate and largely confined to the sub-period at the beginning of which it was fed, while the greatest effect of the phenylacetic acid was seen in a later sub-period. One may infer from this that either the benzoic acid is more quickly absorbed than the phenyl-

[12] Lusk, "Science of Nutrition," W. B. Saunder and Co., 1919, p. 355.

acetic acid, or that glycocoll is more easily synthesized by the body than is glutamine. This latter inference one may explain on the ground that glutamine not only demands 2 atoms of nitrogen for its synthesis but besides an amine requires also an amide group.

During the seventh day of the experiment the greatest reduction is found in the urea nitrogen. At the beginning of the first 4-hour sub-period 4 g. of phenylacetic acid was ingested. This reduced the urea nitrogen to 44% of the total nitrogen, but caused a still greater fall in urea nitrogen (24% of total nitrogen) during the subsequent 4-hour sub-period. At this time a second dose of 6 g. of phenylacetic acid was ingested by the subject, but the percentage of urea nitrogen rose to 29%, only to fall 4 hours later to the very low value of 12% of the total nitrogen. This value was considerably lower than had been previously obtained. McCollum[8] found that the urea nitrogen fell to only about 20% of the total nitrogen after the feeding of large doses of benzoic acid to a pig, and that larger doses of the acid caused only an increase in protein metabolism and a subsequent increase in total nitrogen excreted. In this case, however, the entire 24 hours was considered as a unit. On the seventh day of our experiment it will be seen that the urea nitrogen for the entire 24 hours formed 28% of the total nitrogen.

The ammonia nitrogen was little, if at all, affected by the feeding of either of these organic acids. It has been previously shown by McCollum that an organic acid is detoxicated at the expense of the urea nitrogen, and an inorganic acid (hydrochloric acid) at the expense of the ammonia nitrogen.

It seemed probable that the amino nitrogen for glutamine synthesis would be taken from the urea fraction, but just possible that the ammonia nitrogen might be required to build the amide portion of the molecule. That this is not the case is shown by the fact that there is no decrease in ammonia nitrogen after the ingestion of 10 g. of phenylacetic acid on the last day of the experiment.

Creatinine was unaffected by the acid ingestion, for the creatinine value remained particularly constant during the last days of the experiment.

Lewinski[1] found that 10 to 20 g. of benzoic acid ingested by a man and quantitatively converted into hippuric acid may remove as much as 35% of the total nitrogen in the form of glycocoll (hippuric acid) nitrogen. Ringer[6] obtained much the same results with goats. Wiechowski,[5] employing guinea pigs, found that glycocoll nitrogen might run as high as 64% after the feeding of benzoic acid. After feeding the 6 g. of benzoic acid on the fourth day of our experiment 10% of the total nitrogen was found in the form of hippuric acid nitrogen. On the fifth day 19% of the total nitrogen appeared in the form of glutamine nitrogen. On the sixth day after feeding 3.3 g. of phenylacetic together with 3 g. of benzoic acid, 6% of the total nitrogen appeared as glycocoll nitrogen and 11% as glutamine nitrogen. On the seventh day 35% of the total nitrogen was excreted in the form of glutamine nitrogen, which is comparable to figures found by some of the other investigators. Only in certain sub-periods of certain days does the excretion of glycocoll and glutamine nitrogen reach the value quoted by Wiechowski. In the first sub-period (3 hours) of the sixth day after the ingestion of 3.3 g. of phenylacetic acid and 3 g. of benzoic acid, 29% of the total nitrogen was found in the form of glycocoll nitrogen and 29% in the form of glutamine nitrogen, or a total of 58%. Again in the fourth sub-period (4 hours) of the seventh day after the ingestion of 10 g. of phenylacetic acid 52% of the entire nitrogen output of the sub-period appeared in the urine as glutamine nitrogen.

Moderate doses of benzoic acid are probably excreted quantitatively in the urine as hippuric acid. We found 89% to 93% so excreted within 6 hours after a 3g. dose. Phenylacetic acid seems to require about twice as much time as benzoic acid for its excretion (in the form of phenylacetyl glutamine). Its detoxication, moreover is apparently not so complete, for though after a dose of 10 g. we were able to recover 95% of it from the 24-hour sample of urine, still in other cases after small doses we recovered only about 78% of the amount fed.

Apparently the body builds both glutamine and glycocoll simultaneously as easily and efficiently as either of the compounds alone. After the ingestion of 3 g. of benzoic acid 92% of it was converted into hippuric acid within 6 hours. After the ingestion of 3.3 g. of phenylacetic acid on another day 44% of it was converted into phenylacetyl glutamine within 6 hours. When 3 g. of benzoic acid and 3.3 g. of phenylacetic acid were ingested simultaneously 89% of the benzoic acid and 46% of the phenylacetic acid were changed into their respective detoxication products within a period of 6 hours.

Summary

It has been shown that man will synthesize glycocoll at the expense of urea as do the lower animals. The synthesis of another amino acid (glutamine) at the expense of urea nitrogen has also been demonstrated in the case of a man. The two amino acids may be built simultaneously as readily as either compound alone.

During the period in which these amino acids were being synthesized in the organism, urea nitrogen dropped from about 75% of the total nitrogen to 28%, and during a sub-period of a certain day, to the extremely low value of 12% of the total nitrogen.

After feeding a moderate dose of benzoic acid (3.3 g.), glycocoll for its

detoxication is built within 6 hours, while for the detoxication of a corresponding dose of phenylacetic acid a somewhat longer period of time is required for the synthesis of glutamine.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

THE REACTION BETWEEN ALKALIES AND CERTAIN NITROCYCLOPROPANE DERIVATIVES

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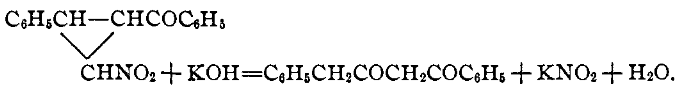
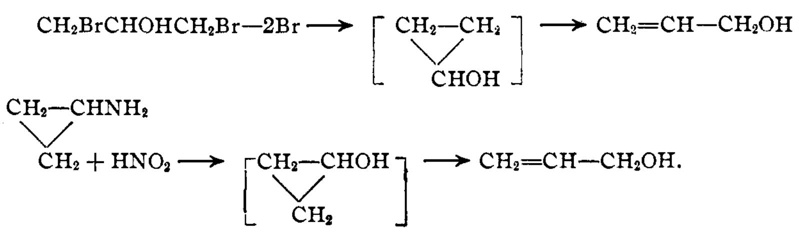
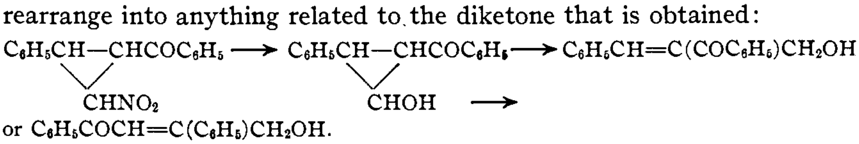
The action of alkalies on all known cyclopropane derivatives is peculiar. A typical reaction is that between phenyl-benzoyl-nitrocyclopropane and potassium hydroxide which gives potassium nitrite and an open chained β-diketone.[1]



Since the reaction involves the elimination of the nitro group, it is conceivable that the first step in the process might be the replacement of this group by hydroxyl. This would give as the primary product a cyclopropanol derivative; but it is probable that cyclopropanols are unstable and like the corresponding ethylenic compounds immediately undergo rearrangement, for when zinc removes bromine from dibromo-*iso*propyl alcohol the product is not the cyclopropanol which would be expected but allyl alcohol,[2] and when cyclopropyl amine is treated with nitrous acid the product is likewise the unsaturated and not the cyclic alcohol.[3]



A cyclopropanol obtained by replacing the nitro group with hydroxyl would therefore probably be unstable, but it would not be expected to rearrange into anything related to the diketone that is obtained:



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[1] THIS JOURNAL, **41,** 1383 (1919).

[2] *J. prakt. Chem.*, **46,** 158 (1892).

[3] *Zentr.*, **76,** [1] 1709 (1905).