THE CHEMISTRY OF GLUCONEOGENESIS.

V. THE RÔLE OF PYRUVIC ACID IN THE INTERMEDIARY METABOLISM OF ALANINE.

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Pyruvic acid occupies a singularly important position in intermediary metabolism. Because of its chemical relationship to alanine and lactic acid

CH_3	CH_3	CH_3
$CH-NH_2$	CO	$CH-OH$
$COOH$	$COOH$	$COOH$
Alanine	Pyruvic acid	Lactic acid

it is assumed to play a rôle in the intermediary metabolism of both protein and carbohydrates.

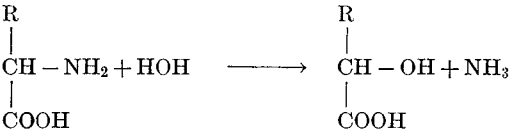
Not until recent years was any attempt made to study the paths that the amino-acids undergo in the process of their catabolism. All that was known was that the amino-acid broke down into a nitrogenous fraction that gave rise to urea and ammonia, and a "nitrogen-free" fraction which was "burnt."

With the development of our knowledge of the structural composition of the amino-acids and their related compounds, evidence began to accumulate which suggested definite reactions and definite paths of decomposition. Until the researches of Neubauer[2] came to light, it was the current belief that the α-amino-acids suffered deaminization in the animal body by a process of hydrol-

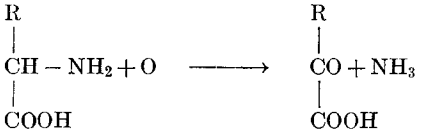
[1] Aided by a grant from the Rockefeller Institute for Medical Research.

[2] O. Neubauer: *Deutsch. Arch. f. klin. Med.*, xcv, p. 211, 1909.

ysis, whereby the NH_2 was removed and an hydroxyl took its place.



Neubauer was the first to call attention to a different process of deaminization, *i.e.*, oxidative deaminization,

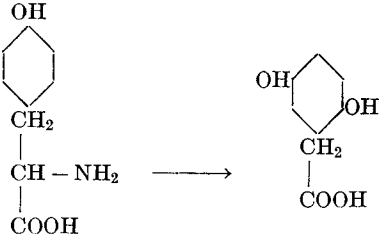


which has so much experimental evidence to support it that it is now almost universally accepted.

We shall not attempt to present a detailed account of the experiments which led to this conclusion, but will briefly state the facts that have a bearing on our present discourse.

Neubauer worked on a patient suffering from alkaptonuria. Such a patient presents an abnormality in his protein metabolism, which consists of his inability to burn tyrosine and phenylalanine and of the excretion of homogentisic acid in the urine. Neubauer and his associates utilized this fact in their study of the intermediary metabolism of tyrosine and phenylalanine[3] in the course of which they came to the following conclusions:

I. That tyrosine (para-oxyphenyl-α-amino-propionic acid) gives rise to extra homogentisic acid[4]



[3] O. Neubauer and W. Falta: *Zeitschr. f. physiol. Chem.*, xlii, p. 81, 1904.

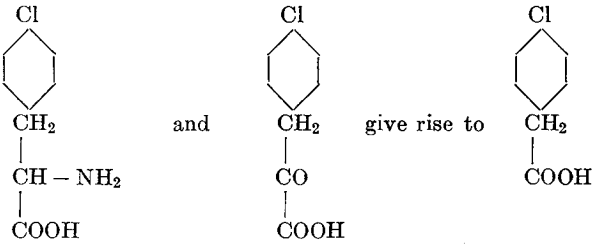
[4] Wolkow and Baumann: *ibid.*, xvi, p. 270, 1892.

II. That para-oxyphenyl-α-hydroxy-propionic acid, $HO.C_6H_4.CH_2.CHOH.COOH$, does not give rise to extra homogentisic acid.

III. That para-oxyphenyl-α-keto-propionic acid, $HO.C_6H_4CH_2.CO.COOH$, gives rise to extra homogentisic acid.

Since these α-amino and α-keto compounds can give rise to homogentisic acid, and the α-hydroxy cannot, Neubauer concluded that the α-amino compound cannot possibly give rise to the α-hydroxy acid as an intermediary body. He then conceived of the "oxidative deaminization" theory, which seems to explain his findings beautifully.

Additional support to this theory was rendered by Friedmann and Masse[5] who showed that



Para-chlorphenyl-α-amino-propionic acid | Para-chlorphenyl-α-keto-propionic acid | Para-chlorphenyl acetic acid

whereas para-chlorphenyl-α-hydroxy-propionic acid, $Cl.C_6H_4.CH_2.CHOH.COOH$, did not give rise to para-chlorphenyl acetic acid. This again showed that the hydroxy acid could not possibly have been an intermediary compound in the metabolism of the amino-acid.

On the strength of his findings Neubauer was led to extend his theory to the entire series of α-amino-acids and suggested that alanine, in the animal body, gives rise to pyruvic acid, which may secondarily give rise to lactic acid.

Reviewing the evidence in support of this theory, one feels convinced of the soundness of the conclusion in the case of the aromatic compounds. With regard to the open chain α-amino-acids, however, there seems to be little direct evidence. It is true that the conversion of α-keto-acids into alanine by the ani-

[5] Friedmann and Masse: *Biochem. Zeitschr.*; xxvii, p. 97, 1910.

mal organism has been proven and that ketonic acids may in the animal body go over with great ease into the corresponding hydroxy acids, which process has been shown repeatedly to be reversible; still we feel that *it is not proven satisfactorily that alanine in its catabolism must pass through pyruvic acid, and that lactic acid can arise only secondarily from pyruvic acid.*

We raise this question because in experiments which we have performed with the object of ascertaining the degree to which pyruvic acid can give rise to extra glucose in diabetic animals, it was found that pyruvic acid does not behave in the way it would if it really occupied a definite and obligatory place in the intermediary metabolism between alanine and lactic acid.

During the course of our work we have come across several substances whose gluconeogenetic properties are not constant, which have the power of yielding more glucose at one time and less at other times, and we have been inclined to attribute their gluconeogenetic properties to certain factors of equilibrium, which turn the reaction of their metabolism in one path at one time and in another path at another time. According to our experience pyruvic acid may be classed among this group.

The status of pyruvic acid in metabolism has been the subject of very considerable discussion during the past year. Parnas and Baer[6] perfused the liver of the tortoise with Ringer's solution, blood and oxygen to which 4 grams of pyruvic acid as sodium salt had been added, and found no increase in the glycogen. On the basis of this one experiment they drew the conclusion that pyruvic acid cannot be classed among the glucogenetic substances. In our estimation, this conclusion is not at all justified.

P. Mayer[7] administered 7 to 8 grams of pyruvic acid to normal rabbits and found that they developed glucosuria, the severity of which depended upon the state of nutrition of the animal. In one rabbit he obtained 2.4 grams of glucose in the twenty-four hours following the administration of pyruvic acid. In animals which had fasted for periods of ten to eleven days, the administration of pyruvic acid was not followed by glucosuria, but by an increase of the glucose concentration of the blood and by an increase in the glycogen in the liver. In this communication Mayer left open the question of the origin of the glucose. In a second paper

[6] Parnas and Baer: *Biochem. Zeitschr.*, xli, p. 386, 1912.

[7] Paul Mayer: *ibid.*, xl, p. 441, 1912.

on the subject[8] (which appeared after our work was far advanced) Mayer studied the influence of pyruvic acid on gluconeogenesis in phlorhizinized dogs and rabbits. He found that pyruvic acid did not give rise to any extra glucose, and that in some cases the urinary constituents were greatly diminished after the pyruvic acid administration. In experiment X the glucose elimination dropped from 13.48 grams in the fore period to 1.45 grams (!) in the experimental period, and the nitrogen dropped from 2.92 grams to 0.53 gram (!). In experiment XI the glucose elimination dropped from 32.93 grams to 3.41 grams (!). The kidneys of these animals were examined microscopically and extensive tubular degeneration was found. Mayer concluded that pyruvic acid is a toxic substance which causes a disturbance in the sugar and nitrogen elimination and which acts by decreasing the permeability of the kidneys.

To all of these conclusions we object most emphatically. We gave Kahlbaum's pyruvic acid seven times to six different dogs in quantities varying from 8.8 to 13.2 grams, administered subcutaneously and orally, and never have we obtained any of the toxic symptoms described by Mayer. In no case did we get the peculiar drop in the glucose and nitrogen eliminations, and in no case did we observe any sign or symptom of any kidney involvement. We have, however, seen a picture of Mayer's experiences with pyruvic acid after subcutaneous and, under certain circumstances also, after oral administration of tartaric acid.[9] The resemblance is so close that we do not hesitate for a moment to attribute Mayer's results to a contamination of his pyruvic acid with tartaric acid. This is all the more probable since pyruvic acid is very largely prepared by the distillation of tartaric acid.

In all of our experiments pyruvic acid appears to be a glucose-yielding substance, the question is only one of degree. The methods employed in these experiments are the same as those employed and described in the previous papers of this series.

In experiment XXII period VIII, 10 grams of pyruvic acid as sodium salt were administered subcutaneously. The glucose elimination rose from 33.6 grams in the fore period to 40.91 grams in the experimental period, and returned to 35.75 and 32.54 grams in

[8] Paul Mayer: *ibid.*, xlix, p. 486, 1913.

[9] Underhill: this *Journal*, xii, p. 115, 1912. We have been able to corroborate Underhill's findings. Our results will be published soon.

the after periods IX and X respectively. The amount of extra glucose eliminated was 8.21 grams.

In experiment XXIII period III, 10 grams of pyruvic acid as sodium salt were given subcutaneously. The glucose elimination rose from 28.54 to 32.04 grams and returned to 22.84 grams in the after period. The D:N ratio, which was 3.44 in the fore period, rose to 4.18 and came down to 3.6 in the after period. The amount of extra glucose was 5.09 grams.

In experiment XXIV period III, 13.2 grams of pyruvic acid as sodium salt were given subcutaneously. The glucose elimination rose from 17.58 grams to 19.18 grams. The amount of extra glucose eliminated was 2.25 grams.

In experiment XXV period IV, 8.8 grams of pyruvic acid as sodium salt were given subcutaneously. The amount of extra glucose eliminated was 5.1 grams.

In experiment XXVI period V, 8.8 grams of pyruvic acid as sodium salt were given subcutaneously. The amount of extra glucose eliminated was 1.16 grams.

In two other experiments, 10 grams of pyruvic acid as sodium salt were given *per os*. The dog vomited part of the ingested material each time. The animals lived for a long time afterwards and no change in the urinary constituents was noticed. The experiments are not reported in detail because the vomitus contaminated part of the urine.

From all these experiments we see very clearly that pyruvic acid cannot be considered a toxic substance (in Mayer's sense) and that in most of the cases pyruvic acid yields large quantities of extra glucose.

However, when we come to compare the results obtained after pyruvic acid administration with those obtained after alanine[10] and lactic acid,[11] we note a very marked difference. While alanine and lactic acid never fail to yield large quantities of extra glucose, pyruvic acid at times yields very small quantities (experiments XXIV and XXVI). This fact makes it very certain that *in the process of catabolism alanine cannot have pyruvic acid as its principal product of intermediary metabolism, and that alanine does not undergo oxidative deaminization.* Alanine and lactic acid yield glucose in quantities so similar to each other, that one seems

[10] Ringer and Lusk: *Zeitschr. f. physiol. Chem.*, lxvi, p. 106, 1910.

[11] Mandel and Lusk: *Amer. Journ. of Physiol.*, xvi, p. 129, 1906.

justified in concluding that the conversion of the former into the latter is quantitative.

In a previous communication[12] it was shown that malic acid yields glucose in quantities similar to aspartic acid, and it was suggested that the former was an intermediary product in the metabolism of the latter. It becomes of interest to know what rôle, if any, the corresponding ketone—oxalacetic acid—plays in it. These experiments are in progress and will be reported soon.

EXPERIMENT XXII. *Twelve-hour periods.*

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D : N	EXTRA GLUCOSE	NH_2N	ACETONE AND ACETO-ACETIC ACID	REMARKS
Feb.									
2	IV		9.82	38.38	3.91		0.89	1.25	
3	V		9.12	33.60	3.68		0.88	1.04	
3	VI	17.40	8.04						
4	VII		8.00						
4	VIII	17.00	**8.65**	**40.91**	**4.73**	**8.21**	**0.505**	**0.308**	10 gms. pyruvic acid as Na salt given subcutaneously.
5	IX		9.18	35.75	3.89		0.50	0.279	
5	X	16.64	8.62	32.54	3.77		0.49	0.283	

EXPERIMENT XXIII. *Twelve-hour periods.*

3	II	12.40	8.28	28.54	3.44			0.340	
3	III		**7.66**	**32.04**	**4.18**	**5.09**		**0.160**	10 gms. pyruvic acid as above.
4	IV		6.34	22.84	3.60			0.430	
4	V	11.54	6.13	23.38	3.81			0.501	
5	VI		5.81	20.51	3.52			0.840	
5	VII	11.20	5.32	20.08	3.77			0.514	
6	VIII		5.21	18.40	3.53				

[12] Ringer, Frankel and Jonas: this *Journal*, xiv, p. 539, 1913.

EXPERIMENT XXIV. *Twelve-hour periods.*

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D : N	EXTRA GLUCOSE	NH_3N	ACETONE AND ACETO-ACETIC ACID	β-OXY BUTYRIC ACID	REMARKS
Apr. 30	I		4.77	18.45	3.87		0.27	0.07	0.24	
May 1	II		5.58	17.58	3.16		0.36	0.11	0.47	
1	III	8.00	**5.26**	**19.18**	**3.65**	**2.25**	**0.36**	**0.13**	**0.39**	13.2 gms. pyruvic acid as above
2	IV		5.74	18.75	3.27		0.29	0.10	0.40	
2	V	7.98	4.47	15.00	3.36		0.19	0.13	0.49	

EXPERIMENT XXV. *Twelve-hour periods.*

May 1	I	9.31	3.77	12.63	3.35			0.22	1.21	
2	II		3.85	12.10	3.14		0.41	0.39	2.14	
2	III	9.11								
3	IV		**3.34**	**13.80**	**4.14**	**5.10**	**0.23**	**0.18**	**0.69**	8.8 gms. pyruvic acid as above.
3	V	8.94	3.30	11.60	3.52		0.23	0.35	1.82	
4	VI		4.40	13.10	2.98		0.35	0.60	2.56	

EXPERIMENT XXVI. *Twelve-hour periods.*

May 16	IV		7.80	28.60	3.68			0.13	0.35	
16	V	12.44	**8.35**	**32.30**	**3.87**	**1.16**		**0.09**	**0.34**	8.8 gms. pyruvic acid as above.
17	VI		7.82	29.51	3.78			0.22	1.00	

SUMMARY.

I. Experiments on phlorhizinized dogs have shown that pyruvic acid is capable of yielding extra glucose in the diabetic organism.

II. In some cases the quantity of glucose was found to be much less than arises from similar amounts of alanine and lactic acid.

III. It is concluded that pyruvic acid cannot be considered a necessary intermediary product in the conversion of alanine into lactic acid and that alanine cannot be considered to undergo oxidative deaminization.