THE CHEMISTRY OF GLUCONEOGENESIS.[1]

IV. THE FATE OF SUCCINIC, MALIC AND MALONIC ACIDS IN THE DIABETIC ORGANISM, WITH CONSIDERATION OF THE INTERMEDIARY METABOLISM OF ASPARTIC AND GLUTAMIC ACIDS, PROLINE, LYSINE, ARGININE AND ORNITHINE.

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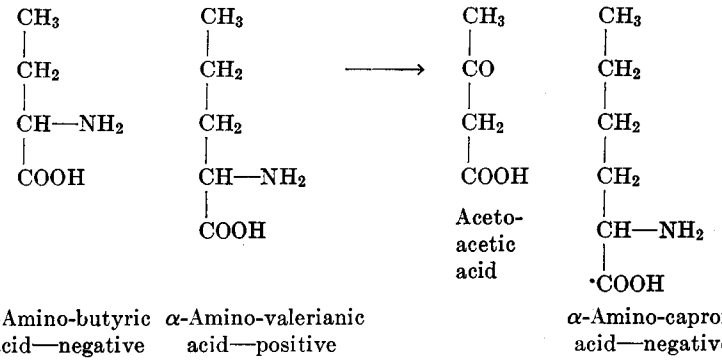
Embden and Marx[2] found that on perfusing an extirpated liver with blood to which α-amino-valerianic acid had been added, there was an increase in the aceto-acetic acid formation, while α-amino-butyric acid and α-amino-caproic acid gave negative results.



α-Amino-butyric acid—negative

α-Amino-valerianic acid—positive

α-Amino-caproic acid—negative

It is evident that α-amino-valerianic acid must have been changed to a four-carbon compound before it could possibly give rise to aceto-acetic acid. The authors therefore concluded that in α-amino-acids the α-carbon, containing the amino radical, becomes

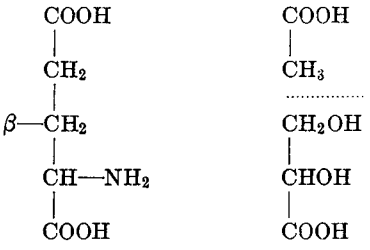
[1] Aided by a grant from the Rockefeller Institute for Medical Research.

[2] Embden and Marx: *Hofmeister's Beiträge*, xi, p. 318, 1908.

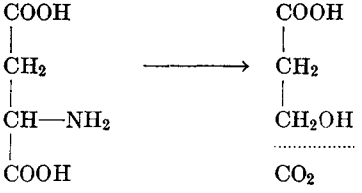
oxidized to a carboxyl state, giving rise to a fatty acid with one carbon less.

Lusk[3] found that the feeding of 20 grams of glutamic acid was followed by an elimination of 13.5 grams of extra glucose.

Ringer and Lusk[4] extended these investigations and found that 20 grams of aspartic acid yielded as much as 14.9 grams of extra glucose. These results corresponded to the conversion into glucose of three of the carbons of either the aspartic or glutamic acid molecule. It was then suggested that glutamic acid undergoes the following changes in the diabetic organism by giving rise to glyceric acid, which was found capable of forming extra glucose.



For aspartic acid it was suggested that the molecule may suffer oxidation in the α-position, *i.e.*, in the carbon containing the amino radical, giving rise to hydracrylic acid.

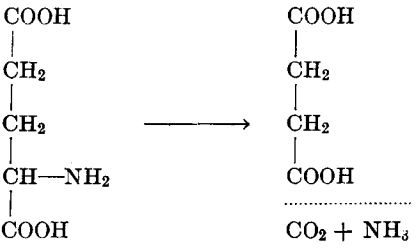


In this series of researches it was our object to test experimentally the paths that these amino-acids may take in their catabolism. In view of the outcome of Embden and Marx' experiments, it suggested itself that glutamic acid may undergo deaminization with oxidation in the α-carbon giving rise to a

[3] Lusk: *Amer. Journ. of Physiol.*, xxii, p. 174, 1908.

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

dibasic acid with one less carbon, *i.e.*, with four carbons, namely, succinic acid. If this were true, succinic acid would yield glucose in the diabetic animal as readily as does glutamic acid.



In experiment XVI, period IV, 11.8 grams ($\frac{M}{10}$) of succinic acid as sodium salt were given *per os*. The glucose elimination, which was 27.26 in the fore period and 25.02 in the after period, rose to 32.74 grams. The D:N ratio rose from 3.41 to 3.99. The amount of extra glucose eliminated was 5.15 grams. In experiment XVII, period XIII, 11.8 grams of succinic acid as sodium salt were given subcutaneously. The yield of extra glucose was much larger, amounting to 9.45 grams.

These two experiments prove very conclusively that succinic acid can yield large quantities of extra glucose. The question now arises—is it merely an incident that these two substances give rise to glucose or does it actually *prove* that glutamic acid passes through succinic acid in its intermediary stages of metabolism? We believe that the latter is the case, and that glutamic acid *does* give rise to succinic acid for the following additional reason: Succinic acid is found as one of the by-products of alcoholic fermentation. Pasteur[5] proved its presence conclusively and found that the quantity of succinic acid bore a relationship of 0.4 to 0.7 per cent to the fermented glucose. He believed that succinic acid was a product of the fermentation of the glucose molecule. This theory of Pasteur greatly disturbed the then current conception of Gay-Lussac that a molecule of glucose, in the process of fermentation, breaks down to two molecules of alcohol and two molecules of carbon dioxide.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

[5] Pasteur: *Compt. rend. Acad. Sci.*, 1858 to 1859; *Ann. de chim. et de phys.*, (3) lviii, p. 323, 1860.

During the next forty years this was the subject of a great many investigations and slowly evidence accumulated which tended to disprove Pasteur's theory of succinic acid arising from the glucose molecule. It was shown that the quantity of succinic acid was not proportional to the amount of glucose fermented, but to the length of time that fermentation was permitted to go on. Buchner and Meisenheimer[6] finally succeeded in showing that succinic acid was not a product of glucose fermentation, but a product of the metabolism of the yeast cell. Working with expressed cell-free juice of yeast, they could find no succinic acid. The problem of the origin of succinic acid in the by-products of alcoholic fermentation was solved definitely by Felix Ehrlich.[7] He found that the higher alcohols of fusel oil were the products of the protein metabolism of the yeast cell. He then devoted his researches to finding the mother substances of succinic acid. It was already known at that time that bacteria were capable of effecting deaminization of amino-acids by splitting off the NH_2 radical and converting it into NH_3 substituting a hydrogen for the removed NH_2.

$$COOH-CH_2-CHNH_2-COOH + H_2 = COOH-CH_2-CH_2-COOH + NH_3$$

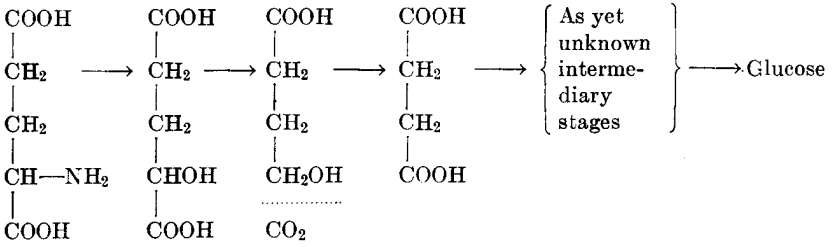
It was also known that in addition to succinic acid small quantities of aspartic acid were found in the fermentation mixture. It therefore suggested itself to Ehrlich that aspartic acid might be the mother substance of succinic acid and that glutamic acid might be the mother substance of glutaric acid, which would mean, if proven, that yeast cells, like bacteria, are capable of bringing about deaminization in a very simple way—by splitting off NH_2 and substituting a hydrogen for it. When he came to subject these ideas to the test of experimentation, he found that the addition of aspartic acid to fermenting yeast and sugar was followed by no increase in the succinic acid, but when glutamic acid was added, he was able "*stets sehr beträchtliche, den normalen Gehalt weit übersteigende Mengen von Bernsteïnsäure, zu isolieren.*"

Here we see the direct transformation of glutamic acid into succinic acid by a living cell, and when we compare these results

[6] Buchner and Meisenheimer: *Ber. d. deutsch. chem. Gesellsch.*, xxxix, p. 3201, 1906; xxxiv, p. 1529, 1901.

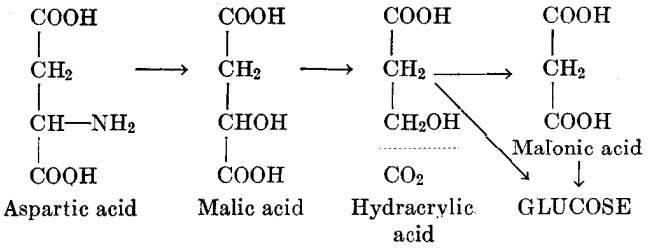
[7] Ehrlich: *Biochem. Zeitschr.*, xviii, p. 391, 1909.

with our own in the diabetic organism, we feel fully justified in concluding that *the path of glutamic acid in metabolism is through succinic acid* undoubtedly passing through α-hydroxy-glutaric acid and γ-hydroxy-butyric acid as intermediary stages:



The fate of aspartic acid in the diabetic organism.

In the foregoing it was shown that α-amino-glutaric acid (glutamic acid) becomes catabolized to succinic acid, which in turn gives rise to extra glucose. A similar path of catabolism suggests itself for aspartic acid, which is chemically very closely related to glutamic acid.



Aspartic acid, as was shown by Ringer and Lusk,[8] and asparagine, as was shown by Knopf,[9] can give rise to large quantities of glucose. In tracing the possible intermediary compounds, one should find all of them capable of yielding glucose to the same extent as does aspartic acid. The first intermediary product of aspartic acid after deaminization appears to be malic acid. In experiment XVI, period II, 13.4 grams ($\frac{M}{10}$) of malic acid as sodium salt were administered subcutaneously. The amount of glucose

[8] Ringer and Lusk: *loc. cit.*

[9] Knopf: *Arch. f. exp. Path. u. Pharm.*, xlix, p. 123, 1903.

elimination rose considerably, but not all of it can be attributed to the malic acid, because there was a considerable rise in the protein metabolism of the same period. The D:N ratio rose from 3.4 in the fore period to 3.97 in the experimental period. The amount of extra glucose eliminated was 5.94 grams. Very convincing results were obtained in experiment XX, period VII. 13.4 grams of malic acid, neutralized with calculated amounts of sodium and potassium hydroxide, were given *per os*. The glucose elimination rose from 14 grams in the fore period to 21.14 grams, in spite of the drop of the nitrogen metabolism. The D:N ratio rose from 3.12 to 5.27 and returned in the after periods to 3.27 and 3.22. The calculated extra glucose amounted to 8.32 grams.

These two experiments prove very conclusively that malic acid gives rise to glucose to approximately the same extent as does aspartic acid, and that it may be considered a product of aspartic acid metabolism.

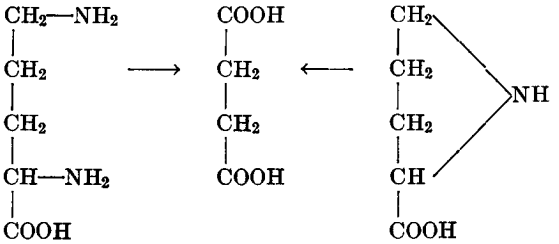
The next question to decide was whether hydracrylic acid and malonic acid give rise to glucose. Unfortunately we had none of the former in our possession and were forced to postpone the experiment to a future date. Malonic acid was administered seven times to five different dogs. It was found that because of the large quantities of alkali necessary to neutralize the acid, it was absorbed with great difficulty, and we were never sure that all of the administered material was absorbed. In some cases it was administered *per os*, in others subcutaneously. The oral administrations were always followed by diarrhoea. The amounts of extra glucose produced by malonic acid are here tabulated:

Experiment	XVII	Period	II	Extra Glucose		0.00	grams.
"	XVIII	"	XVI	"	"	1.00	"
"	XIX	"	IV	"	"	2.64	"
"	XIX	"	VII	"	"	0.65	"
"	XIX	"	X	"	"	2.84	"
"	XX	"	III	"	"	3.06	"
"	XXI	"	III	"	"	1.00	"

The results are very low indeed, but it is very likely that in the transformation of aspartic acid to a compound with three carbons, the reaction ordinarily does not proceed to a point of complete oxidation of the terminal carbon. It is very likely that hydracrylic acid goes over into glucose before the alcohol radical becomes oxidized to a carboxyl.

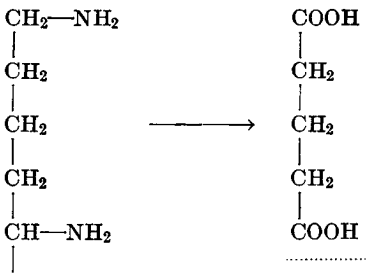
Intermediary metabolism of lysine, arginine, ornithine and proline.

In a recent series of papers, Dakin[10] showed that arginine, ornithine and proline give rise to glucose, when fed to diabetic dogs, while lysine does not give rise to glucose. Dakin rightly suggests that of the arginine only the ornithine moiety goes over into glucose. It seems to us that ornithine and proline give rise to glucose because of their ability to form succinic acid after undergoing deaminization.



Ornithine Succinic acid Proline

Lysine, on the other hand, after undergoing deaminization, becomes converted into glutaric acid and this was shown not to be convertible into glucose.



Lysine Glutaric acid

SUMMARY.

Experiments were performed on phlorhizinized animals.

I. It was found that succinic, malic and perhaps also malonic acids give rise to extra glucose.

[10] Dakin: this *Journal*, xiii, p. 513, 1913; xiv, p. 321, 1913.

II. Evidence was presented to the effect that succinic acid is an intermediary body in the metabolism of glutamic acid, ornithine and proline, which accounts for the conversion of these substances into glucose.

III. It is suggested that malonic acid may arise in part from the catabolism of aspartic acid.

IV. It was also suggested that lysine in its catabolism passes through a glutaric acid stage, which accounts for its non-conversion into glucose.

EXPERIMENT XVI. *Twelve-hour perioas.*

DATE	WEIGHT	PERIOD	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D:N	EXTRA GLUCOSE	NH_3 N	TOTAL ACETONE	REMARKS
Oct. 1912										
30		I	8.35	28.37	1.096°	3.40		0.56	0.28	
30	14.49	II	**10.29**	**40.92**	**1.635°**	**3.97**	**5.94**	**0.29**	**0.33**	13.4 gms. malic acid as sodium salt dissolved in water given subcutaneously.
31		III	7.99	27.26	1.150°	3.41		0.33	0.25	
31		IV	**8.19**	**32.74**	**1.402°**	**3.99**	**5.15**	**0.20**		11.8 gms. succinic acid as sodium salt given *per os.*
Nov. 1		V	7.49	25.02		3.34		0.37	0.367	

EXPERIMENT XVII. *Twelve-hour periods.*

Nov. 1912										
14	12.90	I	3.72	14.38		3.86		0.19	0.56	
14		II	**4.20**	**14.84**		**3.53**	**0.0**	**0.24**	**0.95**	10.4 gms. malonic acid as sodium salt dissolved in water given subcutaneously.
15		III	3.90	14.79		3.79		0.22	0.35	

EXPERIMENT XVIII. *Twelve-hour periods.*

DATE	WEIGHT	PERIOD	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D:N	EXTRA GLUCOSE	NH_3 N	ACETONE AND ACETO-ACETIC ACID	β-HYDROXY-BUTYRIC ACID	REMARKS
April 1913											
7		I	6.14	17.85	0.80°	2.92		0.53			
7	12.53	II	5.53	16.84	0.85°	3.05		0.46	0.47	1.16	
8		III	**5.35**	**24.30**	**1.13°**	**4.55**	9.45	**0.25**	**0.41**	**1.14**	11.8 gms. succinic acid as sodium salt dissolved in water, given subcutaneously.
8	12.46	IV	**4.47**	**16.87**	**0.80°**	**3.78**		**0.28**	**0.37**	**1.46**	
9		V	4.53	15.43	0.72°	3.41		0.33	0.48	1.73	
9	12.71	VI	**4.20**	**15.95**	**0.78°**	**3.80**	1.00	**0.30**	**0.45**	**1.85**	10.4 gms. malonic acid as sodium salt given *per os*. Diarrhoea.
10		VII	3.05	11.21	0.62°	3.68		0.22	0.41	1.56	
10	12.32	VIII	3.11	11.76	0.50°	3.78			0.56	2.34	

EXPERIMENT XIX. *Twelve-hour periods.*

DATE	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D:N	EXTRA GLUCOSE	NH_3 N	ACETONE AND ACETOACETIC ACID	β-HYDROXYBUTYRIC ACID	REMARKS
April 1913											
6	I		4.34	14.22	0.625°	3.28		0.27	0.15	0.39	
7	II		5.10	16.55	0.88°	3.24		0.30	0.22	0.54	
7	III		4.99	16.05	0.75°	3.24		0.28	0.25	0.79	
8	IV	6.80	**5.21**	**19.82**	**0.87°**	**3.81**	**2.64**	**0.23**	**0.41**	**1.56**	10.4 gms. malonic acid as sodium salt given *per os* in one dose. One watery movement of bowels.
8	V		5.08	17.36	0.80°	3.42		0.29	0.52	1.72	
9	VI	6.45	4.99	16.86	0.73°	3.38		0.33	0.48	1.87	
9	VII		**4.93**	**17.45**	**0.78°**	**3.54**	**0.65**	**0.30**	**0.45**	**0.98**	10.4 gms. malonic acid as sodium salt dissolved in water given subcutaneously.
10	VIII	6.43	4.67	16.05	0.73°	3.44		0.27	0.17	0.51	
10	IX		5.28	16.95	0.75°	3.21		0.29	0.17	0.52	
11	X	6.25	**2.88**	**11.83**	**0.55°**	**4.11**	**2.84**	**0.13**	**0.12**	**0.30**	10.4 gms. malonic acid as sodium salt given *per os*. Diarrhoea.
11	XI		3.79	11.46	0.52°	3.03		0.17	0.21	0.63	
12	XII		3.39	9.60		2.83		0.32	0.10	0.26	

EXPERIMENT XX. *Twelve-hour periods.*

DATE	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D:N	EXTRA GLUCOSE	NH_3 N	TOTAL ACETONE	PREFORMED ACETONE	ACETO-ACETIC ACID ACETONE	β-HYDROXY-BUTYRIC ACID	REMARKS
April 1913													
14	I	13.40	7.99	29.35	1.26°	3.67		0.46	0.310	0.061	0.249	1.01	
15	II		8.13	30.41	1.39°	3.74		0.52	0.365	0.059	0.306	1.28	
15	III	13.09	**8.20**	**33.86**	**1.50°**	**4.13**	**3.06**	**0.53**	**0.466**	**0.061**	**0.405**	**1.72**	10.4 gms. malonic acid as sodium and potassium salt given subcutaneously in two doses.
16	IV		9.08	34.39	1.60°	3.79		0.58	0.457	0.083	0.374	1.74	
16	V	12.91	9.42	36.38	1.62°	3.86		0.64	0.554	0.028	0.526	2.39	
17	VI		7.49	28.25	1.32°	3.78		0.47					

EXPERIMENT XXI. *Twelve-hour periods.*

April 15	II		4.43	12.50	0.60°	2.82		0.25	0.206	0.129	0.077		
15	III	10.39	**4.93**	**15.64**	**0.72°**	**3.17**	**1.00**	**0.32**	**0.215**	**0.078**	**0.137**	**0.792**	10.4 gms. malonic acid as sodium and potassium salt dissolved in water given subcutaneously in two doses.
16	IV		4.81	14.90	0.61°	3.10		0.32	0.283	0.087	0.196	1.32	
16	V	10.21	4.32	14.32	0.63°	3.32		0.36	0.271	0 081	0.190	1.86	
17	VI		4.50	14.00	0.60°	3.12		0.45	0.295	0.066	0.229	1.96	
17	VII	9.94	**4.02**	**21.14**	**1.04°**	**5.27**	**8.32**	**0.08**	**0.313**	**0.125**	**0.188**	**1.23**	13.4 gms. malic acid as sodium and potassium salt given *per os*.
18	VIII		3.96	12.92	0.49°	3.27		0.32	0.404	0.123	0.371	2.66	
18	IX	9.77	4.20	13.51	0.60°	3.22		0.31	0.500	0.209	0.291	2.28	