FREE AMINO ACIDS IN ALLOXAN DIABETIC RAT LIVERS

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In the preceding communication (Hohorst et al., 1961) pyruvate and oxaloacetate levels were shown to be lowered in the livers of alloxan diabetic rats due to a higher reduction/oxidation state of the DPN-system and of the DPN-coupled metabolite systems in the C-compartment (Bücher et al., 1959 ; Delbrück et al.,1959) of liver cells. On the other hand malate and glycerol-1-phosphate levels were found to be significantly higher than in non-diabetic controls. Similar alterations have also been observed in livers of adrenalectomized rats after treatment with glucocorticoids. Considering the close metabolic relations of the oxaloacetate- and the pyruvate-systems to glucogenic amino acids, it seemed desirable to extend these investigations to free amino acids. The data available in the literature give only little information on this point. Vest and Wiss (1949) found decreased values of alanine in the liver of alloxan-diabetic rats. Müting (1951) observed lower levels of cystine and methionine in liver and muscle in human diabetes. In the diabetic rat liver the turnover of glycine-C^{14} to glutathione and protein (Krahl,1953) and of methionine-N^{15} to protein (Kritsman et al.,1951) is lowered.

In this communication the levels of 16 amino acids, as determined by means of ion exchange chromatography both in alloxan-diabetic and non-diabetic rat livers, are presented.

Materials and methods

Four perchloric acid extracts were chosen from the diabetic series (D), whose preparation has been described in the preceding paper (Hohorst et al.,1961). Two control extracts of non-diabetic rat livers were prepared with the same technique.

The chromatographic method used represents a scale reduction of the Stein and Moore method (1954 and 1958). The columns consisted of polyethylene tubing of 2 mm inner diameter, filled with Dowex $50^{x}4$ to 3 m length. The fraction volume was reduced to 0,13 ml per fraction according to the smaller diameter of the columns. A micro fraction collector (Schnitger et al.,1959) and extract samples corresponding to 30 mg fresh weight were used. The error of recovery in this method was tested with aid of chromatograms of synthetic mixtures, applying 2×10^{-8} moles of each amino acid to one column; this proved to be $\pm 4\%$ in the case of "acidic" and "neutral" and $\pm 6\%$ in the case of "alkaline" amino acids.

Malate, oxaloacetate, glycerol-1-phosphate, dihydroxyacetonephosphate, ATP, ADP and blood glucose were determined by enzymic tests as described by Hohorst et al. (1959).

Results and discussion

As shown in table 1 the following glucogenic amino acids are lowered in the alloxan-diabetic liver as compared with non-diabetic controls: aspartate, alanine, serine and threonine.To a minor extent glycine and ketogenic phenylalanine and tyrosine also were found to be decreased, whereas leucine, isoleucine, valine, histidine and proline are not or only slightly changed. No conclusive results could be obtained for glutamic acid and lysine due to a large scattering of the values. It is to be mentioned further that the serine peak may include small

Table 1: Levels[+] of amino acids and levels and ratios of other metabolites in diabetic and non-diabetic rat livers.[++]

Number of sample	Diabetes					Control		
	I	II	III	IV	Mean	I	II	Mean
Aspartate	0,19	0,39	0,28	0,54	0,35	2,04	1,90	1,97
Alanine	0,30	0,39	0,49	1,03	0,55	2,20	1,69	2,08
Serine	0,25	0,19	0,37	0,46	0,32	1,80	1,49	1,65
Glycine	0,67	2,16	2,38	1,94	1,79	4,61	3,18	3,90
Threonine	0,04	0,11	0,10	0,18	0,11	0,84	0,59	0,72
Glutamate	0,89	4,10	1,19	4,90	2,78	3,98	3,83	3,91
Valine	0,28	0,34	0,25	0,45	0,33	0,36	0,27	0,32
Leucine	0,18	0,23	0,28	0,29	0,25	0,12	0,22	0,17
Isoleucine	0,16	0,17	0,16	0,20	0,17	0,15	0,16	0,16
Proline	0,20	0,30	0,19	0,20	0,22	0,30	0,23	0,27
Arginine					0,01			0,01
Phenylalanine	0,05	0,04	0,04	0,15	0,07	0,19	0,11	0,15
Tyrosine	0,03	0,03	0,03	0,09	0,05	0,10	0,13	0,12
Histidine	0,24	0,48	0,51	0,43	0,42	0,58	0,52	0,55
Lysine	0,14	0,44	0,21	0,52	0,33	0,63	0,39	0,51
Methionine					0,01			0,01
Pyruvate	0,018	0,019	0,021	0,025	0,021	0,136	0,087	0,112
Oxaloacetate*	2,1	1,2	1,4	1,3	1,5	5,6	6,7	6,2
L/P	107	52	56	91	76	13,4	13,0	13,2
G/D	44	35	20	27	32	8,0	9,0	8,5
M/O	355	450	335	380	380	93	48	70
ATP + ADP	3,51	3,89	3,93	3,77	3,78	3,87	3,88	3,88
Blood glucose**	463	605	513	345	482	85	111	98

[+]The values given are the overall content (micromoles per g fresh weight) of the substance in the tissue, uncorrected for blood = "Gewebsgehalt" (Hohorst et al.,1959); * = millimicromoles per g fresh weight; ** = mg per 100 g

[++]Abbreviations:
L/P = lactate/pyruvate; G/D = glycerol-1-phosphate/dihydroxyacetonephosphate; M/O = malate/oxaloacetate; ATP = adenosinetriphosphate; ADP = adenosinediphosphate.

amounts of asparagine, while the levels of arginine and methionine are too low to permit accurate quantitative determinations in the samples available for chromatography. The

different behavior of the individual amino acids and the constancy of most of the essential ones indicate, that the alterations found do not result from a general alteration of the tissue such as swelling or fat deposition, particularly since the sum of ATP and ADP, which may be considered as a reliable parameter of the cell number in the tissue (Hohorst and Reim, unpublished), was found to be equal in the diabetic and the control livers.

The levels of the amino acids in the controls are in good agreement with results of microbiological assays as published by Wiss (1949). Only the glutamate values are twofold lower than those determined by this author; this may be accounted for by the lack of specifity of the microbiological assay for glutamate, glutamine and glutathione.

While the fact, that one part of the free amino acids is practically unaltered in the diabetic liver, may be regarded as an example of the remarkable stability of the metabolite pattern in living cells under very different metabolic conditions(Bücher,1960), the distinct decrease of another part may be discussed in connection with the alterations of other metabolites. Therefore in table 2 the mean values of aspartate, alanine, serine and threonine levels have been compiled together with the averages of the oxaloacetate and pyruvate levels and the L/P, G/D, and M/O ratios, which have been shown to be altered significantly in the diabetic liver (Hohorst et al.,1961).

As shown in the last column of table 2 the levels of the four amino acids are decreased by nearly the same factor as the reductant/oxidant-ratios are increased in the diabetic group in comparison to the controls. Thus the alterations of these

Table 2

Mean levels and ratios (selected data from table 1)

	aspartate	alanine	serine	threonine	pyruvate*	oxaloacetate**	L/P*	G/D*	M/O*
Diabetes	0,35	0,55	0,32	0,11	0,02	1,5	76	32	380
Control	2,0	2,1	1,7	0,72	0,11	6,2	13	8,5	70
Control/Diabetes	5,6	3,8	5,2	6,5	5,3	4,1	1/5,8	1/3,8	1/5,4

* see also the preceding paper for the corresponding values as determined in a larger sampling

** = millimicromoles per g fresh weight

amino acids seem to parallel those of the oxaloacetate and pyruvate levels; these latter, on the other hand, are dependent on alteration of the oxidation/reduction-state of the DPN-system in the C-compartment of liver cells as shown in the preceding paper. Of special interest is the large decrease of threonine, suggesting that a greater utilisation for gluconeogenesis may result a relative lack of this essential amino acid in the diabetic liver.

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References

Bücher,Th. and Klingenberg,M.; Angew.Chem. 70,552 (1958)

Bücher,Th.; in "Endokrine Regulationen des Kohlenhydratstoffwechsels", 7.Symposion der Deutschen Gesellschaft für Endokrinologie
Springer Berlin - Göttingen - Heidelberg 1961
(in press)

Delbrück,A., Schimassek,H., Bartsch,K., and Bücher,Th.; Biochem.Z. 331,297 (1959)

Hohorst,H.J., Kreutz,F.H., and Bücher,Th.; Biochem.Z. 332,18 (1959)

Hohorst,H.J., Kreutz,F.H., Reim,M., and Hübener,H.J.; Biochem.Biophys.Research Comm. 4, 163 (1961)

Krahl,M.E.; J.Biol.Chem. 200,99 (1953)

Kritsman,M.G., Konikova,A.S., Stepanyan,D.G., and Pyatigorskaya,L.M.; Biokhimiya 16,246 (1951)

Moore,S., Spackman,D.H., and Stein,W.H.; Anal.Chem., 30,1185 (1958)

Moore,S. and Stein,W.H.; J.Biol.Chem. 211,907 (1954)

Müting,D.; Gastroenterologia 86,16 (1956)

Schnitger,H., Papenberg,K.,Ganse,E., Czok,R., Bücher,Th., and Adam,H.; Biochem.Z. 332,167 (1959)

Vest,M. and Wiss,O.; Helv.Physiol.et Pharmacol.Acta 7,C 58 (1949)

Wiss,O.; Helv.Chim.Acta 32,1344 (1949)