Effect of Diabetes on the Concentration of Amino Acids in Plasma and Heart Muscle of Rats

BY RAYMOND SCHARFF AND IRA G. WOOL
Departments of Physiology and Biochemistry, University of Chicago, Chicago, Ill., U.S.A.

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1. The effect of three types of diabetes (alloxan, partial pancreatectomy and anti-insulin serum) on the concentrations of individual amino acids in the plasma and heart muscle of rats was studied. 2. Insulin deficiency produced complex alterations in the concentrations of amino acids in plasma and heart muscle; the concentrations of some (alanine, valine, leucine and isoleucine) increased, others decreased and a small number were unchanged. The complexity of the results may in part be attributed to the diverse hormonal and metabolic changes that accompany diabetes.

Insulin, when added *in vitro* to isolated rat diaphragm or heart muscle, will increase the incorporation into protein of radioactivity from labelled amino acids; the hormone has a similar effect on protein synthesis when administered *in vivo* (for a summary of the evidence see Wool, 1964). In similar circumstances insulin also increases the entry into muscle of most, if not all, of the natural amino acids (Castles & Wool, 1964; Scharff & Wool, 1965*b*). The two effects of the hormone, to increase amino acid transport and to stimulate protein biosynthesis can, however, be shown to be independent of one another. The stimulation by insulin of amino acid transport will occur in the absence of protein synthesis (Castles & Wool, 1964; Scharff & Wool, 1965*b*; Wool & Krahl, 1964), and the stimulation of protein synthesis by the hormone can be shown in circumstances where an increase in amino acid transport is unlikely to have occurred (Manchester & Krahl, 1959; Wool & Krahl, 1959, 1964; Kostyo, 1964).

It remains an important unresolved problem as to whether amino acid transport can ever be the site for the hormonal regulation of protein synthesis in muscle. If protein synthesis is regulated at the transport step, then the accumulation of amino acids in muscle should be decreased in diabetes, for protein synthesis is (Krahl, 1953; Manchester & Young, 1960). However, Castles, Wool & Moyer (1965) found, contrary to expectation, that diabetes increased the accumulation in muscle of ^{14}C from aminoisobutyric acid and from proline, while at the same time protein synthesis was decreased. There is, of course, no assurance that the results obtained in an experiment in which only the accumulation of radioactivity from ^{14}C-labelled amino acids is measured accurately reflect changes in the concentrations of the unlabelled amino acids. For that reason, we have sought to authenticate the findings with regard to the effect of diabetes on the accumulation of radioactivity from ^{14}C-labelled amino acids, by determining the effect of insulin deficiency on the actual concentrations of individual amino acids in heart muscle and plasma.

METHODS

Chemicals. Alloxan monohydrate was obtained from the Eastman Kodak Co. (Rochester, N.Y., U.S.A.); [^{14}C]-inulin (0·23μc/mg.) from the Volk Radiochemical Co. (Skokie, Ill., U.S.A.); glucose oxidase from Worthington Biochemicals Corp. (Freehold, N.J., U.S.A.). The source of the reagents used in the analysis of amino acid was listed by Scharff & Wool (1965*a*). The anti-insulin serum, prepared by immunization of guinea pigs with bovine insulin (Robinson & Wright, 1961), was the kind gift from Dr P. H. Wright; each 1ml. of anti-insulin serum was assayed to be capable of neutralizing 1–1·5 units of insulin. The serum for the treatment of control animals was obtained from guinea pigs that had not been injected with insulin.

Animals. Male Sprague–Dawley rats were maintained under standard conditions (Wool & Krahl, 1959) and allowed free access to food and water at all times. The weight of the animals made diabetic with alloxan or by treatment with anti-insulin serum was 100–140g.; the partially pancreatectomized animals weighed 300–400g. Alloxan-diabetes was induced by the rapid intravenous injection of 60mg. of alloxan monohydrate/kg. body wt. and the animals were used 48hr. later. A group of rats were made diabetic by the intraperitoneal injection of 1·5ml. of anti-insulin serum at 0 and 90min. and the animals killed at 180min. Partial pancreatectomies (Ingle & Griffith, 1942) were performed by Dr D. J. Ingle, and the animals were used 1 month later. The average plasma glucose concentration (in mg./100ml.) of the diabetic

animals (not starved), as determined by the glucose-oxidase (EC 1.1.3.4) method (Huggett & Nixon, 1957), was: alloxan-diabetic, 633 (range 554–690); treated with anti-insulin serum, 233 (range 180–275); partially pancreatectomized, 446 (range 391–524).

Preparation of hearts for analysis. The animals were killed by decapitation, the hearts removed and washed free of blood with 15–20 ml. of Krebs–Henseleit bicarbonate buffer as described by Scharff & Wool (1965*a*).

Analytical procedures. The method of preparation of extracts of heart muscle and plasma, the means of analysis of the extracts by column chromatography for amino acids and other ninhydrin-positive substances, the procedure used to determine the inulin space *in vivo* and the total water content of rat heart muscle were all as described by Scharff & Wool (1965*a*).

Calculations. The means for calculating the inulin space, the intracellular concentration of ninhydrin-positive substances and the concentrations of citrulline and proline when they could not be separated by chromatography were as described by Scharff & Wool (1965*a*).

RESULTS AND DISCUSSION

Alloxan-diabetes was without significant effect on the volume of distribution of [^{14}C]inulin (extracellular space) in rat heart muscle (Table 1). The total water content of the heart, however, was decreased 2% in alloxan-diabetic and partially pancreatectomized rats (Table 1); the decrease is probably a reflection of the dehydration that occurs in diabetes.

In most subsequent experiments, the total water was determined for a portion of the same heart that was analysed for its content of amino acids and the value was used in the calculation of the intracellular concentration. The extracellular water was assumed to be 320 μl./g. wet wt. of tissue, for even brief perfusion (as was necessary to wash blood from the hearts before analysis) will increase the extracellular space to that value (Scharff & Wool, 1965*a*).

The analyses for the concentrations of amino acids in heart muscle and plasma of diabetic rats gave results that were complex. The concentrations of some amino acids increased, others decreased and a small number were unchanged (Tables 2–4). The changes, moreover, were not entirely consistent for the three types of experimental diabetes (alloxan-diabetic, Table 2; partial pancreatectomy, Table 3; anti-insulin serum, Table 4) that were studied. In all three types of diabetes, however, the concentrations in the intracellular water of heart muscle of alanine, valine, isoleucine, leucine and ammonia were increased; the concentrations of threonine, serine and ornithine decreased; the total of all ninhydrin-positive substances was greater than in the controls. In plasma consistent changes in the concentrations of the following were observed: alanine, valine, isoleucine, leucine and taurine were increased; tyrosine and tryptophan were decreased; the total concentration of amino acids and non-amino acids tended to increase.

There was a variation, for the three separate groups of normal controls (Tables 2–4), in the concentrations of amino acids, especially in heart muscle. The reason for the difference is not certain, but there is one possibility. The most striking variations are between the values for the concentrations in heart muscle of the normal controls of Table 3 (pancreatectomy experiment) on the one hand, and those in Tables 2 (alloxan experiment) and Table 4 (anti-insulin experiment) on the other; there is a tendency for the values to be lower in Table 3 (cf. the concentrations in heart muscle of aspartic acid, serine, glycine, valine and ornithine). The determinations of Tables 2 and 4 were for heart muscle from young rats (100–140 g.) whereas those of Table 3 were for heart muscle from older animals (300–400 g.); the higher values for the former might be accounted for by the age difference, since higher concentrations of amino acids are characteristic of tissues from young growing animals (Scharff & Wool, 1964); in conformity with that interpretation the results in Table 3 more closely accord with those previously reported for animals of similar weight (Scharff & Wool, 1964).

An unknown substance, eluted immediately before valine during chromatography, was found in increased concentration in the plasma and heart muscle of alloxan-treated and pancreatectomized rats. The substance was not always detectable in the tissues from normal animals. Though certain identification was not made, the chromatographic mobility of the material suggests that it may be either α-aminobutyric acid or β-aminoisobutyric acid; the two amino acids have been found before

Table 1. *Effect of alloxan-diabetes and pancreatectomy on the inulin space and total water content of rat heart muscle*

For determination of the inulin space, 10 μc of [^{14}C]-inulin/kg. body wt. was administered intravenously to nephrectomized animals 15 hr. before they were killed and the hearts analysed. Further details are given in the text. The values are the means ± S.E.M. of the numbers of observations in parentheses.

Heart muscle from	Water content (μl./g.)	Inulin space (μl./g.)
Normal rats	779 ± 3 (5)	254 ± 6 (5)
Alloxan-diabetic rats	764 ± 3 (5)*	252 ± 7 (5)
Normal rats	792 ± 1 (6)	
Partially pancreatectomized rats	775 ± 7 (5)	

* A difference due to the diabetes that is significant, $P < 0.01$.

in small quantities in rat tissue (Kaplan & Nagareda Shimizu, 1962; Ryan & Carver, 1963), and the latter has been reported to be associated with cellular catabolism (Soupart, 1962).

The complexity of the changes in concentration of amino acids, i.e. an increase of some, while others decrease and a small number are unaltered, and the variability as to the direction of changes for some individual amino acids as between the types of diabetes, may be accounted for by the complicated hormonal and metabolic alterations that characterize insulin deficiency. Certainly, in the diabetic animal an increased secretion of adrenal steroids is to be expected, and the adrenal steroids will influence the mobilization of amino acids from muscle for gluconeogenesis in the liver. Moreover, the adrenal steroids, at least in amounts greater than normal, will influence the accumulation in muscle of amino acids (Wool, 1960; Ryan & Carver, 1963; Kaplan & Nagareda Shimizu, 1963), as well as their concentrations in plasma (Friedberg & Greenberg, 1947; Bondy, 1949; Lotspeich, 1950; Ryan & Carver, 1963; Kaplan & Nagareda Shimizu, 1963). It may be that the changes observed in the alloxan-diabetic and partially pancreatectomized animals were in part the result of a deficiency of insulin and in part due to adrenal steroids acting in an unrestrained manner in the absence of insulin. If that is the case, the alterations that occur in amino acid concentration in the acute reversible diabetes due to the administration of anti-insulin serum may more closely approximate the effects

Table 2. *Effect of alloxan-diabetes on the concentrations of ninhydrin-positive substances in rat heart and plasma*

The animals were made diabetic by treatment with alloxan. The heart muscle from two animals was combined for each analysis. The values are the means ± S.E.M. of 3 (normal) or 6 (alloxan-diabetic) observations. Experimental details are given in the text.

	Concn. in heart (μmoles/100 ml. of intracellular water)		Concn. in plasma (μmoles/100 ml.)	
	Normal	Alloxan-diabetic	Normal	Alloxan-diabetic
Amino acids				
Aspartic acid	617 ± 21·0	625 ± 18·3	3·68 ± 0·78	3·41 ± 0·41
Threonine	127 ± 5·00	95·0 ± 10·6*	41·8 ± 4·96	60·3 ± 2·23
Serine	180 ± 5·97	71·7 ± 4·30***	38·6 ± 2·42	20·4 ± 1·94***
Glutamic acid	1248 ± 47·0	1270 ± 63·0	19·4 ± 1·66	14·2 ± 0·78*
Proline	35·6 ± 3·2	55·3 ± 3·58	29·1 ± 3·22	34·0 ± 2·16
Citrulline	43·2 ± 3·00	34·5 ± 0·45*	13·4 ± 1·43	13·6 ± 0·42
Glycine	167 ± 21·7	117 ± 4·65*	52·2 ± 6·19	31·5 ± 2·43*
Alanine	412 ± 28·2	443 ± 42·3	52·9 ± 1·67	55·6 ± 3·57
Valine	31·3 ± 1·98	62·7 ± 5·31***	19·1 ± 1·56	60·8 ± 3·87***
Cystine (half)			0·95 ± 0·56	0·75 ± 0·21
Methionine	12·7 ± 0·88	13·8 ± 0·40	3·83 ± 0·53	5·85 ± 0·44*
Isoleucine	17·4 ± 1·51	31·2 ± 3·72**	10·6 ± 1·46	28·4 ± 1·63***
Leucine	33·2 ± 2·40	71·0 ± 5·75***	15·3 ± 1·52	48·8 ± 2·71***
Tyrosine	17·3 ± 1·01	17·5 ± 1·44	11·1 ± 2·35	10·3 ± 0·78
Phenylalanine	14·9 ± 0·97	19·3 ± 0·44*	6·79 ± 0·61	10·1 ± 0·58***
Ornithine	13·5 ± 1·90	8·37 ± 0·60*	9·49 ± 1·47	8·94 ± 0·61
Lysine	133 ± 14·9	86·7 ± 4·17*	43·1 ± 6·67	32·9 ± 1·34
Histidine	29·2 ± 1·19	19·3 ± 0·72***	10·2 ± 0·29	11·6 ± 0·55
Tryptophan			7·72 ± 1·24	4·77 ± 0·36
Arginine	60·7 ± 5·48	34·0 ± 1·35***	22·9 ± 1·85	13·5 ± 0·96**
Non-amino acids				
Taurine	5270 ± 168	6620 ± 433*	31·0 ± 3·52	89·1 ± 18·6*
Glutathione	907 ± 125	1100 ± 43·1		
Ammonia	1570 ± 110	1830 ± 97·3	158 ± 19·8	119 ± 6·94
Total				
Amino acids	3244 ± 145	3060 ± 74·4	412 ± 15·9	470 ± 19·7
Non-amino acids	7750 ± 316	9550 ± 488	189 ± 18·1	208 ± 28·8
Ninhydrin-positive substances	10994 ± 404	12610 ± 523	601 ± 27·9	678 ± 26·6

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Table 3. *Effect of partial pancreatectomy on the concentration of ninhydrin-positive substances in rat heart and plasma*

The animals were made diabetic by partial pancreatectomy. The values are the means ± S.E.M. of 6 (hearts) or 4 (plasma) observations. Experimental details are given in the text.

	Concn. in heart (μmoles/100 ml. of intracellular water)		Concn. in plasma (μmoles/100 ml.)	
	Normal	Pancreatectomized	Normal	Pancreatectomized
Amino acids				
Aspartic acid	301 ± 29·4	402 ± 24·1*	3·10 ± 0·51	2·11 ± 1·40
Threonine	156 ± 41·0	102 ± 9·10	34·0 ± 3·19	66·4 ± 7·00*
Serine	100 ± 13·7	59·4 ± 6·13*	37·4 ± 4·21	52·8 ± 6·06
Glutamic acid	990 ± 23·0	893 ± 22·9*	15·9 ± 1·75	12·4 ± 3·51
Proline	36·0 ± 4·30	41·3 ± 1·38	27·8 ± 1·00	72·2 ± 34·7
Citrulline	39·3 ± 4·81	52·8 ± 1·70*	8·51 ± 0·70	25·1 ± 10·9
Glycine	114 ± 20·8	95·0 ± 4·86	39·2 ± 1·75	40·9 ± 3·77
Alanine	436 ± 19·2	500 ± 16·6*	44·5 ± 1·89	76·0 ± 16·6
Valine	23·3 ± 1·39	41·6 ± 5·05**	20·4 ± 0·80	46·5 ± 2·54***
Cystine (half)	6·13 ± 1·09	7·73 ± 0·74	2·44 ± 0·06	6·48 ± 0·76**
Methionine	14·7 ± 1·05	12·0 ± 0·45*	5·98 ± 0·24	7·65 ± 1·17
Isoleucine	17·2 ± 2·71	24·2 ± 3·02	9·08 ± 0·56	18·4 ± 1·14**
Leucine	30·1 ± 5·14	44·6 ± 2·48*	17·3 ± 0·69	37·2 ± 1·49***
Tyrosine	13·4 ± 0·88	10·7 ± 0·63*	10·2 ± 1·05	6·48 ± 1·17
Phenylalanine	10·5 ± 1·72	9·45 ± 0·81	7·91 ± 0·21	7·97 ± 1·42
Ornithine	7·47 ± 0·38	8·80 ± 0·76	7·34 ± 0·38	12·9 ± 1·85*
Lysine	132 ± 7·84	88·8 ± 3·58**	47·6 ± 4·21	44·7 ± 4·73
Histidine	27·4 ± 1·75	21·6 ± 1·59*	7·97 ± 0·81	9·81 ± 0·86
Tryptophan	2·25 ± 0·14	3·58 ± 0·80	10·7 ± 0·58	8·27 ± 0·90
Arginine	63·5 ± 5·61	67·5 ± 2·08	27·2 ± 3·03	35·3 ± 5·98
Non-amino acids				
Taurine	6150 ± 405	6550 ± 431	47·7 ± 5·22	53·7 ± 10·3
Glutathione	1580 ± 177	1210 ± 61·2		
Ammonia	1780 ± 72·0	2150 ± 105*	69·7 ± 5·93	69·2 ± 10·2
Total				
Amino acids	2460 ± 98·1	2480 ± 48·5	390 ± 21·3	587 ± 76·0
Non-amino acids	9510 ± 445	9910 ± 544	117 ± 6·96	123 ± 20·5
Ninhydrin-positive substances	11970 ± 455	12390 ± 508	508 ± 16·5	710 ± 99·8

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

due primarily to a deficiency of insulin. In animals made diabetic with anti-insulin serum the concentrations in plasma of glutamic acid, glycine, alanine, valine, isoleucine, leucine, ornithine, histidine, taurine and ammonia were increased, as were the total concentrations of amino acids and other ninhydrin-positive substances; the concentrations of citrulline, cystine, tyrosine, tryptophan and arginine were decreased (Table 4). The concentrations of ninhydrin-positive substances in heart muscle changed in the following manner: glutamic acid, citrulline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, lysine, histidine, glutathione and ammonia increased; the concentrations of aspartic acid, threonine, serine, tyrosine, phenylalanine, ornithine and taurine decreased (Table 4). The direction of change in concentration was the same in both heart and plasma for the following: glutamic acid, glycine, alanine, valine, isoleucine, leucine, histidine and ammonia were increased; tyrosine was decreased. Since the changes in the concentrations of amino acids in the intracellular water of heart muscle were generally greater than the corresponding changes in the plasma, the concentration ratio (heart/plasma) of the amino acid changed in accord with the direction of the changes in the intracellular concentration (glycine and alanine were exceptions).

The fact that, under certain conditions, insulin increases amino acid accumulation in muscle and that insulin deficiency should have the same effect, at least for some amino acids, is a less surprising

Table 4. *Effect of administration of anti-insulin serum on the concentration of ninhydrin-positive substances in rat heart and plasma*

The animals were made diabetic by treatment with 1·5ml. of anti-insulin serum (1–1·5 units/ml.) by intraperitoneal injection at 0 and 90min., and the animals were killed at 180min.; the control animals were treated with a like amount of non-antigenic serum. The values are the means of 2 (plasma of anti-insulin treated) or 3 observations. Experimental details are given in the text.

	Concn. in heart (μmoles/100ml. of intracellular water)		Concn. in plasma (μmoles/100ml.)	
	Control	Anti-insulin treated	Control	Anti-insulin treated
Amino acids				
Aspartic acid	600	426	5·14	5·47
Threonine†	178	148	41·5	46·4
Serine	152	123*	38·7	40·9
Glutamic acid	1080	1238	15·3	17·5
Proline	38·5	37·8	27·5	26·2
Citrulline	27·8	44·6**	15·1	13·8
Glycine	155	188	44·7	52·6
Alanine	329	375	49·5	60·9
Valine	36·1	45·0	22·6	28·6
Cystine (half)	4·18	4·67	4·48	3·85
Methionine	13·9	17·0	5·93	6·02
Isoleucine	23·1	31·8	12·1	13·7*
Leucine	36·8	55·4	20·4	25·0**
Tyrosine	25·0	15·6*	10·2	8·78
Phenylalanine	20·0	15·4	6·97	6·86
Ornithine	13·5	10·7	9·63	11·0
Lysine	164	178	50·8	53·8
Histidine	22·7	31·8*	9·30	11·4*
Tryptophan	3·35	3·51	9·36	6·39**
Arginine	77·1	75·2	22·2	19·5
Non-amino acids				
Taurine	4030	3460	42·3	56·1
Glutathione	698	1310		
Ammonia	1409	2080	96·2	108
Total				
Amino acids	3230	3060	424	477
Non-amino acids	6140	6850	139	164
Ninhydrin-positive substances	9370	9910	563	641

* $P<0·05$; ** $P<0·01$.

† Includes glutamine and asparagine calculated as threonine.

anomaly when the experimental circumstances are considered. The effect of insulin to increase amino acid transport in muscle is best demonstrated by addition of the hormone *in vitro*, and then with greatest facility when protein synthesis has been inhibited by puromycin (Castles & Wool, 1964; Scharff & Wool, 1965*b*). In those circumstances the effect of the hormone occurs in minutes and, of course, cannot be complicated by secondary changes due to alterations of the titre of other hormones. The effect of diabetes can only be investigated after production of the disease *in vivo*, i.e. only after the passage of days or at the least hours, during which time any number of secondary and complicating metabolic and hormonal alterations may have taken place.

The alterations in concentrations of amino acids in plasma and heart muscle of diabetic animals have proved far more complicated than would have been predicted from the results of experiments *in vitro* with muscle from diabetic animals (Castles *et al.* 1965). It is, for the time, impossible to offer a complete explanation of the means whereby the profound and complex changes are brought about.

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