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The Digestive Release of Amino Acids and their Concentrations in the Portal Plasma of Rats after Protein Feeding

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Attempts to evaluate the nutritional value of proteins have shown that discrepancies sometimes occur between methods based on the amino acid content of the protein and methods involving physiological criteria such as growth performance of animals which have been given the protein. The presence of an amino acid in a protein does not necessarily determine its nutritive value. We therefore attempted to correlate the biological availability of the most limiting amino acids of certain food proteins with their concentrations in the respective proteins. Concentrations of lysine and methionine in plasma from the blood of the vena portae after protein feeding were examined (Guggenheim, Halevy & Friedmann, 1960). The extent and duration of rise of these amino acids in portal plasma was not in complete agreement with the content of the protein sources in these amino acids. The question arose whether this discrepancy is due to different rates of liberation of these amino acids during digestion.

In our previous study (Guggenheim *et al.* 1960) the portal plasma was examined 30, 60, 120 and 180 min. after the protein meal. Maximum concentrations of both lysine and methionine in portal plasma were already observed 30 min. after the meal, indicating rapid digestion and absorption. In the present paper data are presented on the digestive liberation of amino acids and of their concentrations in portal plasma obtained 10 and 20 min. after the test meal.

A further extension of the previous study is the inclusion of tryptophan which, together with lysine and methionine, is one of the most limiting amino acids in cereals, pulses and tubers (Waterlow & Stephen, 1957).

METHODS

The rats used and their diets during the preparatory period have been described by Guggenheim *et al.* (1960). At the end of the preparatory period the rats were starved for 48 hr. This relatively long starvation period was essential for the complete removal of food residues from the intestine. The rats were then given a test meal containing a purified protein with corn starch or fat-extracted soya-bean flour. The test meal consisted of an aqueous suspension of 5% protein and 5% starch, 5 ml./100 g. body wt. being administered by stomach tube. When soya flour containing approximately 50% of protein was used, the suspension contained 10% of the soya flour without starch. Thus each test meal provided 40 mg. of nitrogen derived from the respective protein/100 g. body wt. Controls received a 5% corn-starch suspension without protein. At 10, 20, 30, 60, 120 and 180 min. after the test meal the animals were anaesthetized, the abdomen was opened, and blood withdrawn from the portal vein with a heparinized syringe. The plasma was assayed for lysine and methionine with *Leuconostoc mesenteroides* P-60 and for tryptophan with *Lactobacillus arabinosus*, as described by Barton-Wright (1952). For examination of intestinal contents, clamps were applied at the pyloro-duodenal junction and at the lower end of the ileum. The small intestine was then quickly removed and its contents were obtained by gentle washing of the mucosal surface with water. The intestinal

content was filtered, and the lysine, methionine and tryptophan in the filtrate were determined.

The following protein sources were tested: wheat gluten, zein, casein and lactalbumin (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.). They contained 12·4, 13·6, 13·3 and 10·6 g. of nitrogen/100 g. respectively. For experiments with soya protein the following flours were studied: (*a*) Unheated flour, obtained by ether-extraction of the unprocessed flour with only enough heat to remove excess of solvent; (*b*) commercially processed (toasted) flour; (*c*) overheated flour, obtained by autoclaving flour (*b*) at 15 lb./in.^{2} for 4 hr.

Amino acids in proteins were assayed by the method of Barton-Wright (1952).

RESULTS

Tables 1–3 present data on the amounts of lysine, methionine and tryptophan found in intestine and in portal plasma at various intervals after feeding of protein meals. Widely different patterns of the concentrations of the three amino acids in both the intestine and portal plasma are obtained with different protein sources. Lysine in intestine and in portal plasma increased only slightly after feeding with gluten or zein, and the amounts found in the intestine were even lower than those in controls given starch. Feeding with

Table 1. *Concentrations of lysine in intestine and portal plasma at various intervals after protein meals*

Results are expressed as means with standard errors. The figures in parentheses indicate the no. of animals.

	Lysine in intestine (mg./kg. body wt.)					
Time (min.)	10	20	30	60	120	180
Protein source						
Gluten	6·7±1·6 (8)	8·1±0·9 (8)	7·8±1·3 (8)	7·8±1·5 (8)	5·5±0·7 (9)	6·0±0·8 (8)
Zein	7·6±0·4 (9)	8·3±0·4 (8)	7·2±0·6 (9)	7·2±0·6 (10)	6·0±0·6 (9)	6·9±1·1 (9)
Casein	39·6±4·1 (8)	48·3±8·1 (9)	35·6±5·3 (9)	30·3±3·1 (8)	9·8±1·0 (9)	8·2±0·8 (7)
Lactalbumin	18·9±1·5 (7)	22·2±1·8 (7)	30·6±1·6 (7)	44·3±5·5 (7)	41·1±3·4 (8)	19·7±2·2 (8)
Soya flour (unheated)	17·2±2·3 (10)	24·5±4·3 (8)	29·6±3·5 (10)	25·0±3·3 (12)	21·4±2·6 (9)	11·5±1·9 (10)
Soya flour (processed)	16·9±2·4 (10)	32·7±4·0 (10)	39·6±6·2 (11)	38·1±5·6 (10)	37·7±5·2 (10)	24·2±4·2 (9)
Soya flour (overheated)	5·0±0·2 (9)	5·7±0·4 (8)	5·7±0·4 (7)	7·4±0·8 (8)	8·1±0·9 (5)	10·9±0·9 (8)
None (starch)	10·2±2·6 (8)	10·2±1·4 (7)	10·6±2·2 (8)	8·9±2·6 (8)	6·1±1·0 (8)	6·1±0·6 (7)
None (starvation)	4·5±1·0 (15)	—	—	—	—	—
	Lysine in portal plasma (μg./ml.)					
Time (min.)	10	20	30	60	120	180
Protein source						
Gluten	95±4·9 (6)	95±5·0 (6)	106±3·3 (8)	78±7·5 (6)	71±3·2 (6)	78±3·5 (6)
Zein	92±2·6 (11)	81±5·6 (8)	81±3·8 (7)	72±3·4 (7)	77±2·3 (6)	72±0·8 (6)
Casein	153±4·2 (6)	150±11·5 (6)	175±5·4 (8)	126±5·7 (7)	117±5·6 (6)	95±9·8 (6)
Lactalbumin	94±3·8 (9)	106±3·3 (10)	120±2·9 (9)	103±4·0 (10)	98±2·6 (9)	87±2·8 (10)
Soya flour (unheated)	75±6·4 (7)	71±3·4 (10)	85±7·5 (9)	77±4·5 (11)	75±5·0 (10)	77±2·7 (9)
Soya flour (processed)	72±2·7 (6)	110±3·7 (8)	113±4·6 (7)	104±2·8 (7)	88±4·6 (7)	75±4·3 (6)
Soya flour (overheated)	71±3·7 (6)	74±4·9 (6)	71±1·2 (7)	72±4·0 (10)	71±5·7 (6)	71±5·4 (9)
None (starch)	77±3·7 (6)	78±4·2 (6)	76±2·0 (6)	73±5·8 (5)	75±5·1 (5)	69±3·0 (6)
None (starvation)	75±0·5 (15)	—	—	—	—	—

Table 2. *Concentrations of methionine in intestine and portal plasma at various intervals after protein meals*

Results are expressed as means and standard errors. The figures in parentheses indicate the no. of animals.

	Methionine in intestine (mg./kg. body wt.)					
Time (min.)	10	20	30	60	120	180
Protein source						
Gluten	1·8±0·29 (8)	2·5±0·45 (8)	2·5±0·38 (8)	2·6±0·94 (8)	1·6±0·21 (8)	1·6±0·20 (9)
Zein	1·5±0·17 (8)	1·4±0·13 (9)	1·3±0·15 (9)	1·7±0·20 (10)	1·2±0·11 (7)	1·2±0·11 (10)
Casein	6·8±0·92 (8)	7·2±0·61 (9)	5·8±0·70 (9)	5·3±1·14 (7)	1·8±0·40 (10)	1·2±0·34 (10)
Lactalbumin	3·3±0·22 (7)	3·2±0·15 (7)	4·6±0·20 (7)	6·7±0·78 (7)	6·7±0·44 (7)	3·0±0·56 (8)
Soya flour (unheated)	1·5±0·16 (9)	2·2±0·19 (7)	2·6±0·24 (10)	2·8±0·54 (12)	2·2±0·31 (10)	1·4±0·17 (8)
Soya flour (processed)	2·4±0·43 (8)	4·8±0·92 (8)	5·6±0·84 (11)	6·3±2·13 (8)	7·2±1·39 (7)	3·2±0·78 (8)
Soya flour (overheated)	0·9±0·17 (9)	0·9±0·19 (9)	0·8±0·21 (8)	1·1±0·12 (8)	1·1±0·23 (8)	1·0±0·18 (7)
None (starch)	1·4±0·14 (9)	1·6±0·25 (8)	1·4±0·42 (6)	1·2±0·18 (8)	0·8±0·05 (8)	1·0±0·15 (8)
None (starvation)	0·9±0·15 (17)	—	—	—	—	—
	Methionine in portal plasma (μg./ml.)					
Time (min.)	10	20	30	60	120	180
Protein source						
Gluten	12±0·7 (6)	13±1·7 (7)	20±1·2 (7)	15±1·3 (6)	15±1·3 (7)	12±1·4 (7)
Zein	8±1·0 (8)	9±0·7 (6)	10±0·8 (6)	7±0·1 (6)	7±0·7 (6)	7±0·7 (6)
Casein	22±3·8 (6)	22±1·8 (6)	28±1·9 (7)	19±2·7 (8)	18±2·3 (7)	17±1·5 (8)
Lactalbumin	14±0·6 (6)	12±0·8 (6)	19±1·6 (8)	17±1·4 (6)	17±1·6 (7)	12±0·9 (6)
Soya flour (unheated)	10±1·9 (7)	12±2·8 (6)	11±1·8 (6)	10±1·1 (5)	9±1·4 (6)	7±0·3 (7)
Soya flour (processed)	12±2·4 (5)	12±1·8 (5)	14±2·0 (5)	12±1·0 (7)	11±0·9 (7)	9±1·2 (7)
Soya flour (overheated)	9±0·7 (8)	9±0·9 (10)	9±0·7 (9)	9±0·9 (10)	8±0·6 (7)	8±0·7 (7)
None (starch)	11±1·4 (8)	11±0·7 (6)	14±1·4 (9)	11±1·2 (10)	12±1·6 (8)	12±1·6 (6)
None (starvation)	9±0·7 (18)	—	—	—	—	—

casein or lactalbumin resulted in much higher concentrations in both intestine and portal plasma. Administration of these plant proteins generally produced smaller elevations of methionine and tryptophan concentrations in the intestine than did the animal proteins. In portal plasma the lowest figures for methionine were found after zein and the highest after casein; the smallest rise of tryptophan was observed after zein and the highest after lactalbumin. Methionine and tryptophan concentrations in portal plasma after feeding with zein were generally somewhat below those found in controls that had been given starch.

Of special interest are the results obtained with unheated and processed soya flours, since they contained similar amounts of lysine, methionine and tryptophan. Feeding with untreated flour induced a smaller rise in the concentrations of the three amino acids in both the intestine and portal plasma than did the processed flour. The difference appears to be largest with lysine in portal plasma and with tryptophan in intestine. Overheating

soya flour, which diminished the microbiological availability of amino acids *in vitro*, depressed considerably both enzymic release of the three amino acids and their concentrations in portal plasma. The figures for lysine in portal plasma were even lower than those in the starved rats; this difference, however, is not statistically significant.

Maximum values of amino acids in portal plasma were generally obtained not later than 30 min. after the protein meal; with some proteins and amino acids the peak was reached earlier, e.g. lysine after zein, and tryptophan after gluten, zein or processed soya. Peak concentrations in the intestine, however, were scattered over the whole observation period, and they differed from one protein and amino acid to another. Thus maximum release of tryptophan from casein was found after 10 min., from soya after 30–120 min. and from lactalbumin after 60–120 min. The liberation of amino acids from overheated soya flour was delayed, maximum concentrations being found only after 120 (tryptophan) or even 180 min. (lysine).

For none of the three amino acids was there a

Table 3. *Concentrations of tryptophan in intestine and portal plasma at various intervals after protein meals*

The results are expressed as means with standard errors. The figures in parentheses indicate the no. of animals.

	Tryptophan in intestine (mg./kg. body wt.)					
Time (min.)	10	20	30	60	120	180
Protein source						
Gluten	1·5±0·23 (8)	2·4±0·53 (8)	2·0±0·38 (8)	2·2±0·38 (8)	2·0±0·30 (9)	1·5±0·18 (8)
Zein	1·4±0·10 (10)	1·5±0·06 (9)	1·4±0·15 (7)	1·4±0·16 (8)	1·3±0·09 (10)	1·2±0·10 (8)
Casein	5·4±0·41 (7)	5·2±0·38 (7)	4·0±0·34 (7)	3·4±0·64 (8)	1·4±0·15 (9)	1·2±0·14 (7)
Lactalbumin	3·4±0·29 (7)	4·0±0·26 (7)	5·5±0·43 (7)	9·9±1·21 (7)	9·9±0·92 (7)	3·7±0·60 (7)
Soya flour (unheated)	1·1±0·15 (8)	1·1±0·23 (6)	1·5±0·21 (6)	1·0±0·18 (6)	1·3±0·26 (6)	0·9±0·13 (6)
Soya flour (processed)	3·5±1·34 (11)	6·1±1·08 (11)	7·7±1·28 (12)	8·9±2·40 (11)	9·0±1·87 (10)	4·7±1·17 (10)
Soya flour (overheated)	1·1±0·20 (8)	1·1±0·12 (8)	1·4±0·16 (7)	1·9±0·30 (8)	2·7±0·49 (8)	1·7±0·33 (9)
None (starch)	1·7±0·30 (7)	1·8±0·27 (7)	1·6±0·21 (8)	1·3±0·16 (9)	0·9±0·18 (9)	0·9±0·10 (8)
None (starvation)	0·7±0·06 (14)	—	—	—	—	—
	Tryptophan in portal plasma (μg./ml.)					
Time (min.)	10	20	30	60	120	180
Protein source						
Gluten	22±1·1 (10)	31±1·4 (7)	30±2·3 (6)	24±1·2 (9)	22±1·0 (7)	22±1·4 (6)
Zein	20±1·8 (8)	23±2·2 (7)	23±1·3 (6)	19±1·4 (6)	18±1·1 (6)	18±1·6 (6)
Casein	22±1·6 (6)	22±2·8 (6)	30±1·1 (9)	30±1·5 (6)	20±1·2 (7)	18±1·7 (7)
Lactalbumin	37±1·9 (8)	45±1·9 (8)	52±1·4 (8)	47±2·4 (7)	41±2·2 (6)	39±1·2 (5)
Soya flour (unheated)	24±1·8 (7)	32±3·7 (10)	29±2·4 (6)	27±2·5 (8)	25±2·4 (8)	25±1·5 (7)
Soya flour (processed)	28±2·7 (6)	31±3·2 (6)	36±1·9 (6)	29±1·5 (6)	26±0·8 (6)	25±1·4 (6)
Soya flour (overheated)	24±2·3 (12)	25±2·2 (11)	26±2·3 (9)	30±3·0 (5)	28±2·2 (6)	28±2·8 (5)
None (starch)	21±2·8 (8)	21±1·4 (8)	22±1·6 (7)	22±1·8 (11)	22±1·9 (6)	22±1·2 (11)
None (starvation)	18±0·8 (16)	—	—	—	—	—

Table 4. *Lysine, methionine and tryptophan contents of proteins and the mean increases of these amino acids in intestine and portal plasma after protein meals*

The amino acid contents of proteins are given as mg. of amino acid/100 mg. of nitrogen. The mean increases in the intestine are expressed as mg./kg. body wt. and those in portal plasma as μg./ml.

Protein source	Lysine content of protein	Methionine content of protein	Tryptophan content of protein	Mean increase of lysine*		Mean increase of methionine*		Mean increase of tryptophan*	
				In intestine	In portal plasma	In intestine	In portal plasma	In intestine	In portal plasma
Gluten	8·9	5·7	4·8	2·9	12	1·2	6	1·2	7
Zein	0·4	7·4	0·3	2·7	4	0·5	−1	0·6	2
Casein	45·1	14·7	9·8	24·1	61	3·8	12	1·9	6
Lactalbumin	51·0	12·5	11·5	25·0	26	3·7	6	5·0	26
Soya flour (unheated)	38·9	7·3	7·7†	16·9	2	1·2	−1	0·4	7
Soya flour (processed)	39·4	7·4	7·7	26·9	19	4·0	3	5·0	11
Soya flour (overheated)	31·9	6·2	7·6	2·6	−3	0·1	0	0·9	9
None (starch)	—	—	—	4·2	0	0·3	3	0·6	4

* The correlation coefficients (r) of the mean increases in intestine and in portal plasma were calculated to be 0·59 and 0·57 (not significant) for lysine and methionine respectively, and 0·71 (significant) for tryptophan.

† The amount of tryptophan in untreated soya flour could not be determined owing to inhibition of the enzymic release of amino acids. It was, therefore, assumed to be identical with that in the processed flour.

close correlation between its concentration in the protein and either the extent or the duration of the rise in its concentration in either the intestine or portal plasma. Further, the liberation of lysine and of methionine during digestion correlated only very approximately with the increased concentrations in portal plasma. This follows from Table 4, where the concentrations of the three amino acids in the proteins studied are compared with their mean increases in the intestine and portal plasma over those in the starved animal. The concentration of each amino acid after starvation was selected as a base-line for assessing the increase rather than that following a starch meal since a small rise was observed after feeding starch. The method of calculation of the mean increase may be illustrated by that for tryptophan in portal plasma after lactalbumin feeding. The concentrations after 10, 20, 30, 60, 120 and 180 min. were 37, 45, 52, 47, 41 and 39 μg./ml. respectively (mean 44 μg./ml.), and that in the starved rats 18 μg./ml. Hence the mean increase is $44 - 18 = 26$ μg./ml. The correlation coefficients (r) of the mean increases of each amino acid in intestine and portal plasma were calculated (Table 4); values of 0·59, 0·57 and 0·71 were obtained for lysine, methionine and tryptophan respectively, only that for tryptophan being statistically significant.

DISCUSSION

This study confirms previous results (Guggenheim *et al.* 1960) in showing that there is no close relationship between the concentrations of lysine and methionine in certain food proteins and the extent and duration of the rise in the concentrations of these amino acids in portal plasma after a protein meal. The figures of the present study differ somewhat from the corresponding values of the previous investigation, but they are of the same order of magnitude. The previous observations have been extended to include a third amino acid, namely tryptophan, which may limit the nutritive value of proteins. The reason for the lack of a complete agreement was sought in differences in the rate and extent of the release of the amino acids during digestion. Here again a certain relationship was found, but the correlation between intestinal and portal amino acid concentrations was statistically significant for tryptophan only. As pointed out by Guggenheim *et al.* (1960), the extent and duration of the elevation of the concentration of an amino acid in portal plasma reflect the concentration of the respective amino acid in the food protein and its liberation during digestion as well as the rate and extent of its absorption.

The amounts of amino acids liberated in the intestine and consequently their concentrations in

portal plasma after feeding with zein were very low. This is consistent with the very slow rate of digestion of zein *in vivo* (Geiger, Courtney & Geiger, 1952; Gupta, Dakroury & Harper, 1958; Rogers, Chen, Peraino & Harper, 1960).

The nature of the effect of heat-treatment on the nutritional defect of untreated soya-bean flour is uncertain in spite of much experimental work on the subject. Our results are consistent with the findings of other workers (Jacquot, Matet & Fridensen, 1947; Riesen, Clandinin, Elvehjem & Cravens, 1947; Evans & McGinnis, 1948; Hou, Riesen & Elvehjem, 1949; Sheffner, Adachi & Spector, 1956), who found a depression of the rate of liberation of amino acids from unheated as well as from overheated soya protein in digestion tests *in vitro*. Moreover, proper heat-treatment results in improved absorption of amino acids from the small intestine of rats (Carroll, Hensley, Sittler, Wilcox & Graham, 1953). Severe heat-treatment may even lead to partial destruction of amino acids, lysine being particularly sensitive (Evans & Butts, 1948).

Intestinal absorption of amino acids appears to proceed rapidly. Maximum concentrations in portal plasma were generally observed 30 min. after the protein meal and quite often before peak concentrations were reached in the intestine. The decrease in the concentration of an amino acid in portal plasma at a time when its concentration in intestine continues to rise (e.g. lysine after lactalbumin, methionine and tryptophan after processed soya meal, tryptophan after lactalbumin) remains unexplained.

The lack of a close relationship between the release of an amino acid during digestion and its concentration in portal plasma, as exemplified by lysine and methionine, may be due to absorption mechanisms. Amino acids are believed to be transported across the intestinal mucosa by a special mechanism (Hoeber & Hoeber, 1937). According to Kratzer (1944) the absorption of amino acids varies inversely with their molal volume. On the other hand, L-amino acids are absorbed more rapidly than the D-isomers (Gibson & Wiseman, 1951), suggesting selective absorption. Further, the presence in the intestine of certain amino acids inhibits the absorption of others (Kamin & Handler, 1952; Pinsky & Geiger, 1952; Hagihara, Ogata, Takedatsu & Suda, 1960). Thus certain amino acids liberated during the digestive process may interfere with the absorption of others, and this may result in different patterns in the intestine and portal plasma.

A small rise in the amino acid concentrations was observed after a starch meal. This may be due to amino acids present in digestive juices and intestinal epithelium which, after being released, are transported by the portal vein. The concentrations of amino acids in the intestine after feeding with starch are generally lower than after the protein meals. This is not in agreement with the reports of Nasset, Schwartz & Weiss (1955) and Nasset (1957). These authors found approximately constant proportions of 15 amino acids in the small intestine whether protein or non-protein test meals were given. Lysine, for example, which is almost absent from zein, was present in the jejunum in approximately the same proportion after feeding with either zein or egg albumin. Our results are not in agreement with these findings.

SUMMARY

1. Young starved rats were given meals composed of 50% protein and 50% carbohydrate, 250 mg. of protein being given by stomach tube per 100 g. body wt. After 10, 20, 30, 60, 120 and 180 min. the animals were killed and the concentrations of lysine, methionine and tryptophan in the intestinal content and portal plasma were determined. The following protein sources were studied: wheat gluten, zein, casein, lactalbumin and soya flour.

2. The rate and extent of the release of the amino acids during digestion were not in close agreement with their respective concentrations in the proteins.

3. Protein feeding was generally followed by a rapid rise of amino acid concentrations in portal plasma. The extent and duration of the increase of tryptophan, but not that of lysine or methionine, showed a significant correlation with the rate and extent of the digestive release in the intestine.

4. Proper heat-processing of soya flour increased both the liberation in the intestine and the concentrations in portal plasma of the amino acids, whereas severe heat-treatment depressed them.

5. The amount of an amino acid present in portal plasma after a protein meal depends not only on its quantity in the food protein and the rate and extent of its release during digestion but also on the rate of its absorption through the intestinal wall.

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Polyol Dehydrogenases

4. CRYSTALLIZATION OF THE L-IDITOL DEHYDROGENASE OF SHEEP LIVER*

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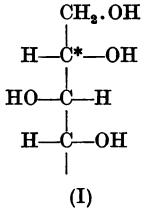
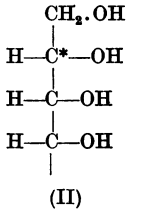
L-Iditol dehydrogenase (sorbitol dehydrogenase) operates specifically with DPN and catalyses the reversible oxidation of several acyclic polyols to ketoses (Blakley, 1951). Fully hydroxylated pentitols, hexitols and heptitols are oxidized if they possess configuration (I) or (II) (McCorkindale & Edson, 1954), C* indicating the site of oxidation.



(I)



(II)

The enzyme occurs in the livers of all mammalian species that have been examined and procedures have been described for significant purification of the enzyme from some species (Williams-Ashman & Banks, 1954; Todd, 1954; King & Mann, 1959; Holzer & Goedde, 1960). Similar enzymic activity has been found in certain male accessory sexual glands (Williams-Ashman, Banks & Wolfson, 1957; Hers, 1957), spermatozoa (King & Mann, 1959) and guinea-pig-liver mitochondria (Hollmann & Touster, 1957). The partially purified enzyme extracted from guinea-pig-liver mitochondria (Hollmann, 1959) has been called 'DPN–xylitol (D-xylulose) dehydrogenase' (Hickman & Ashwell, 1959).

McCorkindale & Edson (1954) showed that substrates of L-iditol dehydrogenase possessing configuration (I) (sorbitol, L-iditol, xylitol) are oxidized substantially faster than those with configuration (II) (allitol, ribitol). It is possible that their rat-liver preparation contained two polyol dehydrogenases, each specific for one of these configurations, but the data of Williams-Ashman *et al.* (1957) provide evidence that a single enzyme is responsible for the oxidation of both types of polyol.

To apply a more exacting test to the unitary hypothesis, an extensive purification of the readily extractable polyol dehydrogenase of sheep liver was undertaken. A preliminary note on the crystallization of the enzyme has been published (Smith, 1960).

MATERIALS AND METHODS

Materials

Source of the enzyme. Sheep livers were obtained at the slaughter-house within 15 min. of the death of the animals and immediately chilled in crushed ice. Livers of starved

* Part 3: Shaw (1956).

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