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STUDIES IN EXPERIMENTAL LATHYRISM—III EFFECT OF LATHYRISM ON THE IMINO ACIDS OF CHICK EMBRYOS

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Abstract—The imino acid pool decreased in chick embryos, which had been treated with β-aminopropionitrile. The proportion of hydroxyproline was not changed. The specific activities of free proline and hydroxyproline after injection of ^{14}C-labelled proline or glutamic acid also decreased, which was confirmed by the study of those imino acids, which had been incorporated into collagen.

ALTHOUGH it is generally agreed that in lathyrism there is a defect in the organization of collagen at macromolecular level,[1] there must be concomitant changes in amino acid metabolism also, either as a cause or as a consequence. Indications of these changes in the level of free amino acids have been mentioned in literature: delayed incorporation of ^{14}C-proline into granuloma slices at incubation in the presence of β-aminopropionitrile,[2] delayed incorporation ^{15}N-labelled ammonium chloride into lathyritic chick embryos,[3] an increased concentration of α-oxo acids in the tissues of lathyritic rats[4] and changes in the amino acids of blood[5] or urine[6] of lathyritic rats. The purpose of the present experiments was to throw more light on the metabolism of the imino acids in lathyritic chick embryos with view on their central position in the synthesis of collagen.

EXPERIMENTAL

Treatment of the chick embryos

Fertilized eggs were hatched for 14 days at +37° temperature and 80 per cent relative humidity. β-Aminopropionitrile (10 mg per embryo as 10% aqueous, neutralized solution of hydrosulfate) was injected either into the yolk sac or on the chorio-allantoic membrane. The control eggs received the corresponding volume and amount of sodium sulfate. Simultaneously was also injected 0·5–1·0 μc (in one μmole) of the radioactive amino acid (^{14}C-proline, ^{14}C-glutamic acid or ^{14}C-leucine). The labelled amino acids were supplied by the Radiochemical Centre, Amersham, England.

Preparation of the samples

The whole embryos were homogenized in 1 M NaCl solution, buffered with 1/15 M phosphate to pH 7·4, using a Potter-Elvehjem homogenizer (Type B as specified in the catalogue of Arthur H. Thomas & Co.). Following preparations were obtained:

Soluble diffusible supernatant by dialysis of the homogenate against distilled water. The diffusate was concentrated at water bath;

Insoluble collagen: the total homogenate in 1 M NaCl was centrifuged at 53,000*g* for for 60 min and the sediment was extracted with 0·1 M sodium citrate buffer, pH 3·8. The residue was suspended in water and gelatinized for 3 hr at +120° in autoclave. The supernatant was collected by centrifugation and evaporated at water bath;

Total collagen: the whole embryos were homogenized into 1% NaCl solution. The homogenate was centrifuged and autoclaved as above but without extraction with citrate buffer.

The radioactivities of the amino acids of the supernatant and of the hydrolyzed (overnight in 6 N hydrochloric acid at +100°) collagen preparations were determined from the *DNP-derivatives of the amino acids*, which were synthetized by Sanger's method and isolated by paper chromatography according to Levy (Ref. 7).

TABLE 1. IMINO ACIDS IN DIFFUSIBLE SUPERNATANT OF CHICK EMBRYO HOMOGENATES. C, control; L, lathyritic embryos

Experimental				Imino acids		
No.	Injected*	duration, hr	C/L	μM/embryo	per μM soluble nitrogen	hydroxy-proline, per cent
MP-4	proline	4	C (pooled)	11.8	0.024	26
			L (pooled)	7·5	0·019	22
MP-5	proline	2	C (pooled)	11·3	0·023	20
			L (pooled)	9·1	0·014	24
MG-2	glutamic acid	4	C (pooled)	9·2	0·021	23
			L (pooled)	9·0	0·018	26
MG-3	glutamic acid	4	C (pooled)	9·5	0·021	25
			L (pooled)	9·1	0·019	21
ML-1	leucine	4	C (pooled)	20·6	0·031	20
			L (pooled)	15·4	0·024	22
ML-2	leucine	4	C (pooled)	19·5	0·026	18
			L (pooled)	15·0	0·031	26
MP-6A	proline	4	C_1	19·0	0·028	27
			C_2	18·5	0·029	20
			C_3	17·5	0·028	16
			means	18·3	0·028	
			L_1	16·5	0·023	24
			L_2	20·2	0·025	17
			L_3	18·0	0·022	20
			means	18·3	0·023	
Average of experiments above			C	15·2	0·026	22
			L	13·3†	0·022‡	22
MP-6B	proline	4	C_1	19·3	0·024	19
			C_2	26·3	0·028	17
			L_1	18·8	0·026	20
			L_2	27·0	0·033	15

* In the yolk sac; except in the experiment MP-6B on the chorio-allantoic membrane;
† Probability of the difference $P < 0{\cdot}05$ (data treated as non-independent pairs);
‡ Probability of the difference $P < 0{\cdot}02$ (data treated as non-independent pairs).

Analyses

The imino acids were determined according to Troll,[8] hydroxyproline according to Neuman and Logan[9] and the nitrogen after Kjeldahl combustion and distillation of the ammonia. The DNP-amino acids were estimated from the light absorption of aqueous solution. The molar extinction at 3500 Å was taken to be 16×10^3.

The radioactivities were measured with 'Tracerlab' 16P Gas-Flow counter and results corrected to infinite thickness and expressed as specific activities cpm/μmole.

RESULTS

From Table 1 it can be concluded that the total soluble imino acids were decreased in lathyritic embryos, also on the basis of the soluble nitrogen. The proportional fraction of hydroxyproline, presumably a breakdown product from collagen, seems to be constant. After hydrolysis the hydroxyproline increased by one-fifth but there was no difference between the normal and lathyritic samples. This is in agreement with the observations of Decker *et al.*[9] When the proline was injected on the chorio-allantoic membrane, the imino acid concentrations showed larger variation and the effect of lathyrism was masked. The symptoms of lathyrism were not affected by the administration of proline.

Table 2 shows data on the radioactivities of the free imino acids. The specific activity of proline and also of the hydroxyproline was always lower in treated embryos. The data on imino acids which have been incorporated in collagens confirm this conclusion.

TABLE 2. SPECIFIC ACTIVITIES OF SOME FREE AND PROTEIN BOUND AMINO ACIDS IN CHICK EMBRYOS.

C, Control and L, lathyritic embryos, the specific activities expressed as cpm/μmole.

Experimental				Imino acids			Aspartic and glutamic acids
No.	injected*	duration, hr	C/L	bound or free	proline	hydroxyproline	
MP-4	^{14}C-proline	4	C	free	705	206	19
			L		30	52	9
MP-5	^{14}C-proline	2	C	free	447	17	7
			L		244	69	11
MG-2	^{14}C-glutamic acid	4	C	free	14	34	21
			L		0	19	24
MP-1a	^{14}C-proline	24	C	insoluble	790	280	—
			L	collagen	690	240	—
MG-1	^{14}C-glutamic acid	4	C	total	23	60	—
			L	collagen	7	40	—
MP-3	^{14}C-proline	4	C	insoluble	407	220	—
			L	collagen	77	60	—

* In the yolk sac.

DISCUSSION

It is evident that the total amounts, relative concentrations and specific activities of imino acids are decreased in the supernatant tissue fluid of lathyritic embryos. In earlier experiments with slices[2] it was concluded that the amount of the endogenous proline was decreased. In a living system the conditions are more complicated. As an explanation could be claimed that the resorption of proline into the embryo from outside, *e.g.* from yolk sac, would have been retarded, because of damaged capillaries in lathyrism. This suggestion could not explain, why the proportion of imino acids of all the amino acids is decreased. It can also be doubted whether capillary damage could develop so soon. The lower specific activities of imino acids from insoluble collagen

could also be explained by the decreased formation of insoluble collagen from its soluble precursors.

If the relationship proline–glutamic acid would change in lathyrism to the favor of glutamic acid, proline would never reach so high specific activities in lathyritic embryos as it does in the normal organism, because the turnover of the glutamic acid is very rapid. However, there is no direct evidence for this kind of change.

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