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Conversion of Pyruvic Acid to Alanine in the Silkworm Larva

As reported earlier[1], we tried to isolate keto acids of importance physiologically in the body fluid and the silk-glands of the silkworm larvæ and found that there were present glyoxylic, α-keto-glutaric, oxalacetic and acetoacetic acids. Pyruvic acid, which is found usually in mammals, was not recognized in the silkworms. The present work was carried out to determine whether, although pyruvic acid is not found in the body fluid and the silk-glands, metabolism involving pyruvic acid takes place in the silkworm.

The silk-glands, the alimentary canals, also the muscle and the fat tissues obtained from five larvæ (Si 110 × Nichi 122) on the sixth day of the fifth instar were washed with cold water and suspended in *M*/15 phosphate buffer, *p*H 7·4, and homogenized in homogenizer and made up to 5 ml. Each sample was placed for 5 hr. at 0° and then kept at − 18° overnight. The insoluble proteins obtained by thawing the ice at room temperature were discarded, and the supernatant solution was used as the enzyme solution. To 1 ml. portions of each enzyme solution were added 0·5 ml. of 0·1 *M* L-glutamic acid, 0·5 ml. of 0·1 *M* sodium pyruvate and 0·1 ml. of *M*/15 phosphate buffer, *p*H 7·4, containing 0·1 μc. of sodium pyruvate-2-^{14}C, and this mixture was incubated for 60 min. at 38°. The solutions of L-glutamic acid and sodium pyruvate were made in *M*/15 phosphate buffer, *p*H 7·4, immediately prior to use. The reaction was stopped by adding 200 mgm. of trichloracetic acid. This filtrate was analysed by means of paper chromatography, using *n*-butanol/acetic acid saturated with water (*n*-butanol, 95/acetic acid, 5) as a solvent. Two spots were revealed on a strip which was sprayed with ninhydrin in the usual way: one of them (R_F 0·42) was alanine and the other (R_F 0·16) was glutamic acid remaining in the reaction mixture. The strip was scanned for radioactivity by an *SC*-16 windowless gas-flow counter. As shown in Fig. 1, higher radioactivity was recognized at the position of the alanine. A lower radioactivity was also recognized at the position of R_F 0·14, at nearly the same position as glutamic acid, but this proved, by comparison with the behaviour of pure sodium pyruvate-2-^{14}C on a chromatogram, to be due to the appearance of isotopic sodium pyruvate remaining in the reaction mixture on the chromatogram. Radioactivities of the alanine spot on the chromatograms obtained from three tissues of the silkworm are presented in Table 1. The results seem to suggest that the synthesis of alanine from pyruvic acid with glutamic acid takes place in the silk-glands, the alimentary canal, and muscle and fat tissues. Recently the occurrence of the transamination reaction in the silkworm was also indicated by Bheemeswar *et al.*[2] and Koide *et al.*[3].

Table 1. RADIOACTIVITIES OF THE ALANINE SPOT ON THE CHROMATOGRAMS

Tissues	Counts/min. of alanine spot formed
Posterior division of silk-gland	420
Alimentary canal	330
Muscles and fat tissues	273

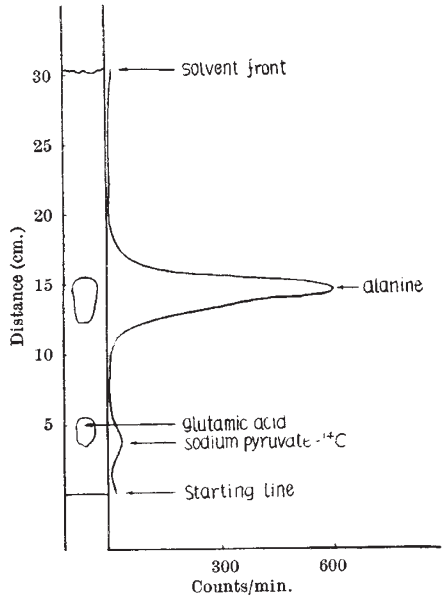

Fig. 1. Radioactivity of chromatogram of reaction mixture *n*-butanol/acetic acid saturated with water run from the starting line

As glutamic acid and aspartic acid are the main constituent amino-acids of the proteins of the mulberry leaves, this reaction, which produces alanine from pyruvic acid with glutamic acid, seems to be of special importance in the synthesis of the alanine (25 per cent) of the silk, as well as in the formation of alanine by β-decarboxylase[4] from aspartic acid in the silkworm. Florkin *et al.*[5] demonstrated, using radioactive phenylalanine-1-^{14}C, that the carboxyl carbon of phenylalanine was not utilized for the synthesis of alanine of the silk by the silkworm. Recently I ascertained that the carbon-14 of the sodium pyruvate-2-^{14}C given to silkworms appeared in the alanine isolated from the cocoon fibres produced by these silkworms, and glutamic acid and aspartic acid played the most important part at the synthesis of alanine from pyruvic acid with amino-acids[6].

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