either protected only against the homologous challenge (*melitensis* serum) or protected better against homologous challenge (*abortus* serum).

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Feb. 17.

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Microbiological Determination of Free Amino-Acids in the Body Fluid of the Silkworm Larva (*Bombyx mori*)

The kinds of free amino-acids present in the body fluid of the silkworm larva have been determined by paper chromatography[1], but little is known of the quantities present. The present work was carried out to determine the concentrations of individual free amino-acid by microbiological assay.

Material was obtained from the silkworm, *Si* 108 (*Kyu*), at the third and sixth days of the 5th instar (51 and 124 hr. from the commencement of the feeding period). The feeding period of the 5th instar was about 148 hr. The method of preparing the samples for microbiological assay was, in principle, that described by Elvehjem *et al.*[2] for the assay of rat blood plasma except for the precipitating agent. The precipitation of the proteins in the body fluid was carried out with trichloracetic acid instead of tungstic acid as a precipitating agent, because free amino-acids in the body fluid, especially some of the basic amino-acids, such as histidine and lysine, which are precipitated with the protein by using tungstic acid according to the procedure of Elvehjem *et al.*, are not precipitated by using trichloracetic acid[3]. 10 ml. of the body fluid which was collected in a tube kept in ice-water was added to a bottle containing 90 ml. of 3 per cent trichloracetic acid, and the bottle and contents allowed to stand at room temperature for 30 min. with intermittent shaking. The precipitates were removed by centrifugation, and the filtrates were heated in a boiling water-bath for a few minutes. The clear protein-free filtrates were adjusted to *p*H 6·8 with *N* sodium hydroxide, made up to 100 ml. with the distilled water and stored in a refrigerator under toluene until determinations were carried out. The microbiological assays on seventeen free amino-acids in the trichloracetic acid filtrates were carried out by the procedures described by Tamura *et al.*[4]. The 100- to 400-fold dilutions of the fresh body fluid were suitable for determining the majority of the amino-acids except histidine (400- to 2,000-fold dilutions).

The concentrations of free amino-acids in the body fluid are summarized in Table 1. Recovery of amino-acids added to the trichloracetic acid filtrates was 90–110 per cent in most cases. The individual free amino-acid concentrations in the body fluid obtained by using tungstic acid as the precipitating agent were, except for the basic amino-acids, in good agreement with those obtained by using trichloracetic acid. The concentrations of the individual free amino-acids in the body fluid on the sixth day of the 5th instar were found; histidine and lysine (more than 1,000 μgm./ml.), were very high; serine, aspartic acid, threonine, arginine, tyrosine, alanine, glutamic acid, valine, glycine, leucine and proline (460–100 μgm./ml.) came next; and phenylalanine, methionine, tryptophan and *iso*leucine (less than 100 μgm./ml.) were comparatively low. A somewhat similar value was also observed in body fluid of the third day of the 5th instar. Except for tyrosine[5], body fluid concentrations of the other individual free amino-acid shown here have apparently not been reported.

Table 1. Concentrations of Free Amino-Acids in the Body Fluid
(Values in μgm. per ml.)

Amino-acids	Body fluid of the silkworm		Organism*	Human blood plasma (Bergeim *et al.*, ref. 7)	Rat blood plasma (Elvehjem *et al.*, ref. 6)
	Third day of 5th instar	Sixth day of 5th instar			
Alanine	305	176	*Cit*		
Arginine	356	210	*M*	16	32
Aspartic acid	60	240	*M*		
Glutamic acid	90	175	*S*		
Glycine	284	129	*M*		23
Histidine	2,199	2,805	*Cit*	14	10
*iso*Leucine	75	46	*M*	13	13
Leucine	153	116	*M*	19	27
Lysine	889	1,042	*M*	22	58
Methionine	97	78	*M*	5	10
Phenyl-alanine	73	96	*M*	10	14
Proline	103	104	*Cit*		43
Serine	201	463	*M*		
Threonine	314	237	*S*	17	44
Tryptophan	32	52	*M*	13	17
Tyrosine	279	202	*M*	10	22
Valine	255	138	*M*	27	27

* *Cit* = *Leuconostoc citrovorum*; *M* = *Leuconostoc mesenteroides*; *S* = *Streptococcus faecalis*.

In general, the concentrations of the individual free amino-acid in the body fluid of silkworm larvæ are strikingly different from those of the rat[6] and of human blood plasma[7] (Table 1); a particular feature is the much higher concentrations of basic amino-acids, such as histidine and lysine.

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Feb. 3.

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Preparation of Metallic Isotopic Targets of the Alkali and Alkaline Earth Metals by Evaporation of their Compounds

Electromagnetically enriched isotopes of the elements lithium, potassium, rubidium, calcium, strontium and barium, after the necessary chemical processing, are only available in one of the standard gravimetric forms, whereas nuclear targets for reaction studies in high-voltage accelerators are