

lysine to the effects already observed with intact proteins^{4,5}. Similar effects were observed in all three systems⁶, confirming the view that the free amino groups of the protein are of great importance in the protein-sugar reaction. Even insolubility, preceded by an increase of molecular weight, occurred in the polylysine experiments, showing that cross-linking can apparently occur without the participation of other amino-acid residues.

This work was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research. A full account will be published elsewhere.

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three sericin fractions. This was not histidine, for although histidine has been reported as present in sericin by other workers we could not detect it in any of the hydrolysates by the sensitive Pauly reaction⁹. The spot we obtained may have been due to hydroxy-lysine; but our evidence regarding this amino-acid was by no means conclusive.

The distribution of the amino-acids in sericin is being investigated by Sanger's technique¹⁰ and by other methods.

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Amino-acids of Silk Sericin

THE silk thread extruded by the larva of the silk moth of commerce (*Bombyx mori*) consists of two parts: a central core of two filaments of the protein fibre 'fibroin', surrounded and cemented together by a layer of protein material called 'sericin'.

Analyses of the amino-acids of sericin are widely scattered through the literature¹⁻⁴, and are in no case claimed to be complete. By the methods of paper-partition chromatography we have examined the amino-acid constitution of sericin, and demonstrated the presence of fifteen amino-acids. Of these, valine had not previously been reported, and the evidence for a number of others, especially lysine, proline, glycine and phenyl alanine, was very slight.

The sericin was divided into three fractions, depending on their solubility in hot water, or in dilute acid or alkali. This separation follows the general lines described by various earlier workers⁴⁻⁶. The outer, most soluble fraction, sericin *A*, had a total nitrogen content of 17.2 per cent; paper chromatography of the hydrochloric acid hydrolysate of this fraction showed it to contain the following amino-acids: serine, threonine, glycine and aspartic acid in high concentration; glutamic acid, arginine, alanine, leucine, isoleucine, valine and tyrosine in moderate concentration; and lysine and phenyl alanine in relatively low concentration.

The second fraction, sericin *B*, had a nitrogen content of 16.8 per cent. On acid hydrolysis it yielded the amino-acids of sericin *A* in similar proportions, and in addition some tryptophane.

The third, inner layer of sericin, sericin *C*, adjacent to the fibroin, was insoluble in hot water, but could be removed from fibroin by treatment with hot dilute acid or alkali. Its nitrogen content was 16.6 per cent and on hydrolysis it yielded proline in addition to the amino-acids of sericin *B*. This inner layer also contained sulphur, present as inorganic sulphide. No sulphur-containing amino-acids could be detected.

There was some evidence for the presence of traces of a third basic amino-acid residue in each of the

Preparation of the Specific Purified Antigen of Canine Hepatitis Virus and Antiserum for Serological Studies

THE natural history of mammalian hepatitis viruses supports the concept of a widespread unapparent infection^{1,2} which makes it extremely difficult to study the problem by experimental inoculations without a reliable serological test. This communication describes a simple method for the purification and concentration (if necessary) of the antigen, as well as the preparation of the active antiserum for serological studies. Four strains of the virus have been used in these experiments: two English strains, one from Australia and one from Sweden.

Preparation of antigen. Methanol cooled to -30°C . was cautiously added to a final concentration of 30 per cent to a cooled fine 10 per cent suspension of a virus-rich tissue in distilled water or to undiluted body fluid (such as serum, peritoneal fluid, etc.), while it was kept in an ice box. The resultant turbid fluid was held at 0°C . for 2-3 hr. and then centrifuged at 4,000 r.p.m. until all the suspended particles were thrown down. The supernatant fluid was removed and the sediment was taken up in a volume of 0.85 per cent saline at pH 6.8-7.0 equal (or less, if desired) to that of the original liquid, and after stirring thoroughly it was allowed to stand for 24 hr. at 0°C . Any undissolved material was then removed by centrifugation and the final water-clear supernatant was used as the antigen. Ethanol or butanol can be used instead of methanol, but the resultant antigens appeared to be less active.

Preparation of antiserum. Dogs were hyper-immunized with a fine 10 per cent suspension (in 0.85 per cent saline) of the virus-rich liver. The intravenous or subcutaneous inoculations of the material used were carried out by giving increasing amounts (1 to 11 ml.) of the suspension over a period of three weeks. In the intraperitoneal method three injections were given (5 ml. each) at weekly intervals.