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THE PROTEIN OF THE SALVARY GLAND SECRETION IN DROSOPHILA

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At the beginning of the prepupal stage, the salivary gland of Drosophila becomes enormously inflated. This inflation is due to the appearance of a large amount of material in the lumen of the gland. Prior to the prepupal stage this material is in the cytoplasm of the gland cells but not in the lumen of the gland. It is undoubtedly the secretion released from the cells into the lumen soon after the larval stage terminates. The origin and movements of this material will be described in detail elsewhere.

If an inflated gland is isolated from the prepupa and its tissue is broken in water, the secretion flows out, and unless the water is distinctly acid it dissolves quickly, forming a viscous solution. If, on the other hand, an inflated gland isolated from a prepupa is placed in 95% alcohol, the secretion immediately hardens and becomes opaque while the tissue remains transparent. The secretion can be easily isolated in this condition by removing the tissue with needles under the dissecting microscope. The isolated secretion retains the same solubility in water as before alcohol treatment provided it is not kept too long in alcohol. If an isolated secretion is transferred to absolute alcohol, it immediately becomes brittle and loses its solubility in water except at high pH's. In its native state, i.e., before alcohol treatment, most of this material does not dissolve in common lipid solvents.

By separating the inflated glands from about 800 prepupae of *Drosophila melanogaster* in isotonic saline and isolating the secretions as completely as possible from the tissues in 95% alcohol, about 1600 pieces of secretion were collected. These were transferred to ether as soon as isolated from the tissues and exhaustively extracted, and the residue was thoroughly dried. From the weight of 600 pieces of the residue, the average weight of one piece of secretion was found to be approximately 4 μg. The ether-soluble

fraction of the secretion was found to be very small in proportion to the residue. Its chemical nature will be described elsewhere and only that of the residue will be reported here.

The residue is insoluble in all common organic solvents and even in dilute or concentrated mineral acids unless heated, but it dissolves readily in weak or strong alkali solutions without heating. A sample of residue was dissolved in dilute NaOH solution and the solution was neutralized with HCl and tested for various color reactions with the following results: positive for ninhydrin and biuret reactions; slightly positive for Molisch reaction; and negative for Millon's, Hopkins-Cole and xanthoproteic reactions. These results suggested that the substance is a protein containing an unusually small amount, if any, of aromatic amino acids. The ultra-violet absorption spectra showed no selective absorption between 210 μ and 310 μ. The absence of selective absorption at about 290 μ agrees with the negative color reactions for aromatic amino acids. The absence of selective absorption at 260 μ indicates absence of nucleic acids in the residue. These tests were repeated with several samples with consistently the same results. The residue is, therefore, not significantly contaminated with the gland tissue, for otherwise the nucleic acid would be detected. In fact, microscopic examinations revealed that the number of cells present in the residue was so extremely small that they can be entirely neglected. It may be added that the dissolved residue is precipitated by phosphotungstic acid but not by trichloroacetic acid.

The nitrogen content of the substance was found by micro-Kjeldahl analysis to be 10.8%. This figure was obtained from one sample of 4.8 mg. and it is unusually low for a protein. When samples of the residue were hydrolyzed with 10% HCl for 24 hours with constant boiling of the acid and when the acid was evaporated on a steam bath, a substantial amount of crystalline inorganic salt was found in the dry residue. Although the proportion of the salt in the samples was not determined, it seems that the unusually low figure for the N content found in the above analysis can well be accounted for by the presence of the inorganic salt, and a large amount of glutamic acid and some glucosamine, both of which have low nitrogen content (see below).

As a preliminary to the two-dimensional chromatogram analysis, three samples of small quantities of the residue were hydrolyzed with 10% HCl for 24 hours with constant boiling of the acid and the hydrolyzates were analyzed on filter paper strips, one with collidine and two with phenol as the solvent. For the principle and technique of the partition chromatogram method of protein analysis, the reader is referred to Consden, Gordon and Martin (1944)[1] and to Dent (1946)[2]. In one of the phenol strips an intensely colored band was present at $Rf = 0.21$. In the control strip in which glutamic acid was used, the Rf of the band was 0.20. In the other phenol strip a strongly colored band was also present at about the same

position. In the collidine strip also an intensely colored band was present at $Rf = 0.12$. In the control strip in which glutamic acid alone was used, the Rf of the band was 0.15. These bands in the experimental and control strips are sufficiently close to each other to indicate that the band in the experimental strip represents glutamic acid. Less intensely colored bands were found at various other positions on all three strips.

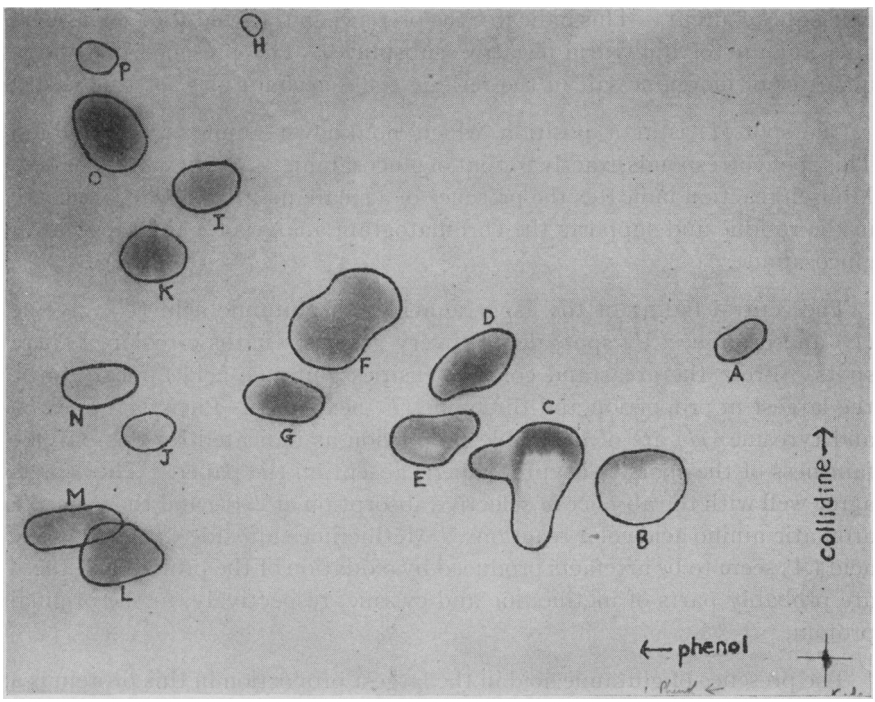

FIGURE 1

The result of a two-dimensional chromatogram analysis of the acid hydrolyzate of the protein in the secretion of the salivary gland in Drosophila is shown. Each circle represents one amino acid or an oxidation product of an amino acid. The hydrolyzate was put at the position of the cross at the lower right and phenol and collidine were run in the respective directions indicated by the arrows. *A*, cysteic acid; *B*, aspartic acid; *C*, glutamic acid; *D*, serine; *E*, glycine; *F*, threonine; *G*, alanine; *H*, tyrosine; *I*, glucosamine; *J*, methionine sulfoxide; *K*, valine; *L*, lysine; *M*, arginine; *N*, proline; *O*, leucine; *P*, phenylalanine.

Since most of the bands on the strips represented mixtures of two or more amino acids, a two-dimensional analysis was made to separate all the amino acids contained in the residue in order to identify them individually. Eight hundred μg. of the residue was taken for this purpose. This is about ten times as much as the amount required for an adequate analysis of an

ordinary protein by the chromatogram method. The residue was hydrolyzed with 10% HCl for 24 hours in a sealed glass tube placed in boiling water and the hydrolyzate was analyzed on a square filter paper with phenol and collidine as solvents. The result is shown in figure 1. Sixteen ninhydrin-positive spots are recognized, fifteen of which are identified as amino acids. The color of these spots is, on the original paper, only about half as intense as would be expected if the sample consisted entirely of a protein of usual N content. This indicates the presence in the residue of a considerable amount of ninhydrin-negative substance. The presence of a large quantity of inorganic salt in the residue could account also for this result.

One spot (*I*) is at a position which no known amino acid occupies. This spot corresponds exactly to that of glucosamine. A very weak, positive Molisch reaction indicates the presence of a small quantity of carbohydrate in the residue and supports the chromatogram analysis for the presence of glucosamine.

The central fading of the aspartic acid (*B*), glutamic acid (*C*), glycine (*E*) and arginine (*M*) spots denotes very great strengths of color of these spots. From the areas and color intensities glutamic acid appears to be the largest in proportion and threonine (*F*) next to it. Phenylalanine (*P*) and tyrosine (*H*) are of negligible proportion as indicated by the extreme faintness of the spots. Tryptophane is absent on the paper. These facts agree well with the absence of selective absorption at 290 μ and the negative aromatic amino acid color reactions. Methionine sulfoxide (*J*) and cysteic acid (*A*) seem to be artefacts produced by oxidation of the protein and these are probably parts of methionine and cystine, respectively, in the original protein.

The presence of glutamic acid in the largest proportion in this protein is a noteworthy characteristic, but not unusual as a number of proteins are known to contain a disproportionately large quantity of this amino acid. The unique feature, however, is the unusually large proportion of threonine (*F*) and serine (*D*). Dr. Dent informs me that he knows of no other protein so far analyzed by the chromatogram method which shows such an intensely colored and broad spot of threonine in relation to the other spots. Although an accurate quantitative determination is not possible, the fact that this spot is so unusually out of proportion indicates an extraordinarily high content of threonine in the protein of the residue. The serine spot is likewise unusually high in intensity in proportion to the other spots, indicating also an extraordinarily large content of this amino acid in the protein.

As pointed out already, this protein together with the ether-soluble substance is secreted into the lumen of the gland as soon as the animal begins to pupate. The secretion remains in the lumen for a short time and disap-

pears. Where it goes and what it is used for is now being investigated. Although no evidence has yet been obtained, the fact that it disappears from the gland at an early pupal stage suggests a possibility that it either enters into the formation of a part of the pupal body or participates in the chemical processes of histogenesis. One possible function it may have would be to provide through digestion a very large amount of glutamine. Glutamine readily enters into transamination reactions and its presence thus would provide an ample source of a variety of amino acids which must be required for the rapid rate of protein synthesis occurring at this stage of development. Similarly, serine which is present in an unusually large proportion in the secretion protein may, after being freed from the protein, become an abundant source of precursors of other amiino acids such as glycine and cystine (Stetten, 1942).[3] As to threonine, which is contained also in an extraordinarily large amount in this protein, its possible use in histogenesis cannot be surmised, since only relatively little is known concerning the metabolism of this amino acid.

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[1] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).
[2] Dent, C. E., *The Lancet*, Nov. 2, 1946.
[3] Stetten, A., *J. Biol. Chem.*, **144**, 501 (1942).

LINKAGE STUDIES OF THE RAT. IX. CATARACT

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Cataract is a dominant character discovered in an albino strain of rats by Smith and Barrantine, and described by them in the *Journal of Heredity*, **34**, 8–10, 1943. A stock of cataractous individuals, 3 females and 2 males, was kindly supplied to us for linkage studies in September, 1943.

No indications have been found of linkage between the gene for cataract and any other gene of the rat. The tests made and their results are summarized in table 1. The progress of the tests has been slow because the cataract is recognizable only in individuals which have unpigmented eyes albinos or pink-eyed yellows. Colored individuals must be subjected to