2 mg/ml of crystalline pepsin. Sections of dog parotid were treated with papain and pepsin while monkey submaxillary was digested in pepsin only. After mild acid hydrolysis and proteolytic digestion, some slides were further incubated in *Cholerae vibrio* neuraminidase for 16 h and then stained in alcian blue and/or colloidal iron.

Glands which underwent treatment with acetate/HCl buffer 2.5 at 75° completely lost their alcian blue or colloidal iron staining. However, when sections of the same group were further digested in pepsin or papain, they showed a homogeneous basophilia throughout the tissues. These results clearly indicated (1) that in the glands examined sialic acid carboxyls are to a great extent free, (2) that a small portion of neuraminic acid cannot be histochemically revealed in the tissue and is not split off from the glycoprotein molecule by the mild hydrolytic procedure. In this small fraction, sialic acid carboxyls appear to be blocked by basic proteins and can only be released after digestion with pepsin (monkey submaxillary) or with pepsin and papain (dog parotid). Finally, basophilia, which returned in the sections after the proteolytic treatment, can be accounted for only by sialic acid, since the subsequent digestion with neuraminidase completely abolished the stainings from the tissues examined.

Riassunto. In questo lavoro si è cercato di dimostrare, con mezzi istochimici, che l'acido sialico presente in tessuti di ghiandole mucose e sierose si trova in due forme differenti. Per la maggioranza tale acido è libero ed il suo gruppo carbossilico interagisce con alcuni coloranti cationici. Una piccola quantità di acido neuramminico è, invece, bloccata da proteine basiche e può essere istochimicamente rivelata solo dopo trattamento con enzimi proteolitici.

G. QUINTARELLI[12]

University of Alabama Medical Center, Birmingham (Alabama, U.S.A.), November 23, 1962.

[12] On leave of absence from the University of Rome Medical School, Viale Regina Elena 287-A, Roma (Italia).

Occurrence of 3,4-Dihydroxyphenylacetic Acid Glucoside in Abdomen of Female Locust, *Locusta migratoria* subsp.

The natural occurrence of the glucoside of *o*-dihydroxyphenol in insects has first been reported by BRUNET and KENT[1]. They have found the glucoside of protocatechuic acid in the left collaterial glands of the cockroaches, *Periplaneta* and *Blatta*. Besides this compound, the glucoside of N-acetyl-dopamine has been isolated from *Drosophila*[2] and *Calliphora*[3].

During the course of studies on the metabolism of phenols in insects, the author has found a new conjugated phenol in the mature female locust, *Locusta migratoria* subsp.

Abdomens of mature female locusts (about 20 individuals) were homogenized with 50 ml of 90% ethanol. After centrifugation the supernatant was evaporated to dryness under reduced pressure in an atmosphere of hydrogen. The residue was taken up with 10 ml of distilled water and shaken with petroleum ether to remove fat. The aqueous layer was passed through the column of ion-exchange resin, Dowex-50 ($\times 8$, H^+, 1×3 cm). The eluate was concentrated to a small volume and shaken with ethyl acetate. Both the ethyl acetate and aqueous fractions were subjected to paper chromatography.

The chromatograms, obtained by using various solvents, showed that the ethyl acetate fraction contains only one phenolic substance, having the same Rf value as that of authentic 3,4-dihydroxy-phenylacetic acid (spot A in Figure 1).

The chromatogram of aqueous fraction showed two spots, the one with the same Rf value as 3,4-dihydroxyphenylacetic acid and the other more conspicuous one with lower Rf value (spot B in Figure 1). It was identified

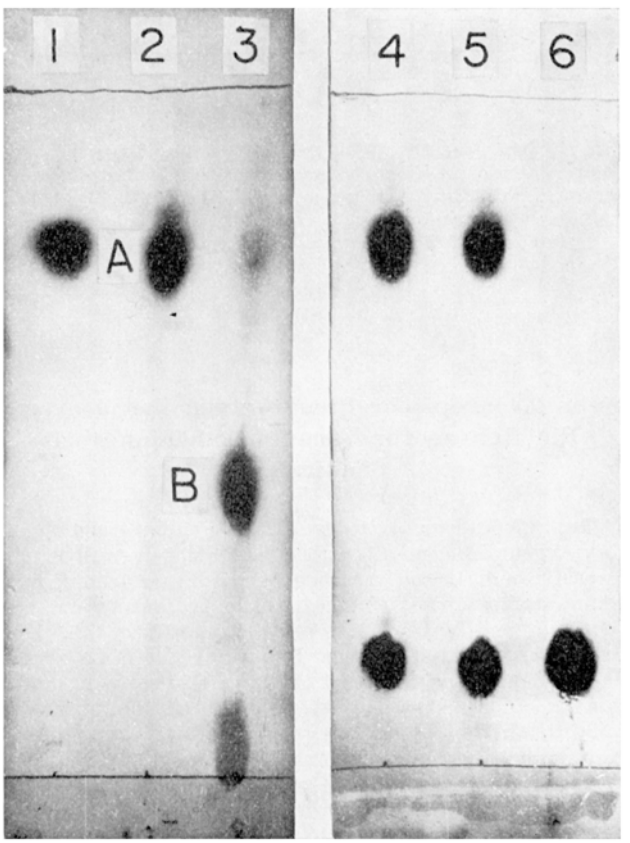

Fig. 1. Paper chromatograms of phenol derivatives found in the mature female locust, and its hydrolysates.—Ascending method was used. The solvent was a mixture of *n*-butanol, acetic acid and water (4:1:2 by volume). Filter paper was Toyo No. 51. Colour was developed with Folin-Ciocalteu reagent (lefthand chromatogram) and ammoniacal silver nitrate (righthand chromatogram).—(1) Authentic 3,4-dihydroxyphenylacetic acid. (2) Ethylacetate fraction. (3) Aqueous fraction. (4) Hydrolysate of spot B with N-HCl. (5) Hydrolysate of spot B with β-glucosidase. (6) D-glucose.

[1] P. C. J. BRUNET and P. W. KENT, Proc. Roy. Soc. B *144*, 259 (1956).

[2] S. OKUBO, Med. J. Osaka Univ. *9*, 327 (1958).

[3] P. KARLSON, C. E. SEKERIS, and K. E. SEKERI, Hoppe-Seylers Z. *327*, 86 (1962).

as the glucoside of 3,4-dihydroxyphenylacetic acid from the following evidence. The spot B gave positive reaction with Folin-Ciocalteu reagent but not with Arnow reagent and ammoniacal silver nitrate. These reactions indicate that the substance in question is not a free *o*-dihydroxyphenol.

Ultra-violet absorption spectrum of the eluate from spot B was very similar to that of 3,4-dihydroxyphenylacetic acid (Figure 2).

Accordingly, the eluate from spot B was hydrolysed with N-hydrochloric acid at 100°C for 3 h and the hydrolysate was subjected to paper chromatography. As shown in Figure 1 (4), two reducing spots were detected. These Rf values corresponded to those of 3,4-dihydroxyphenylacetic acid and glucose respectively.

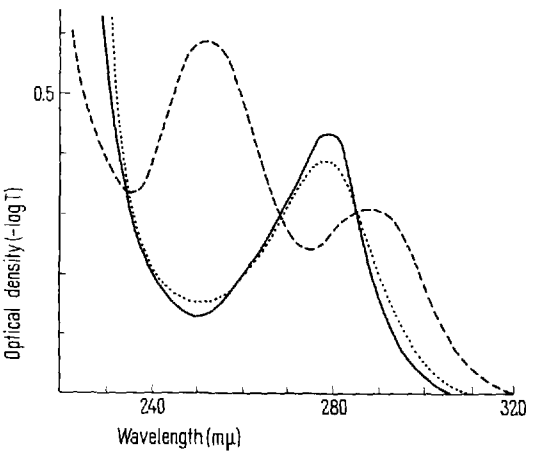

Fig. 2. Ultra violet absorption spectra of phenol derivatives.—All samples were eluted from chromatograms with distilled water and measured with Hitachi Spectrometer Model EPU-2a. Solid line; authentic 3,4-dihydroxyphenylacetic acid. Dotted line; the eluate from spot B. Broken line; authentic protocatechuic acid.

The same result was obtained when the eluate from spot B was incubated at 37°C for 30 min with β-glucosidase prepared from apricot (Figure 1 (5)).

These results provided very strong evidence that the substance B was a β-glucoside of 3,4-dihydroxyphenylacetic acid.

In order to know the position of glycosidic linkage, substance B was mixed with 2,6-dichloroquinone-4-chlorimide in an alkaline solution, following the method of GIBBS[4]. Indophenol blue was formed instantaneously, indicating that the position *para* to the phenolic hydroxyl group is unsubstituted, that is, glucose must be bound to 3,4-dihydroxyphenylacetic acid by 4-hydroxyl group.

Therefore, substance B was assumed to be 3-hydroxy-4-O-(β-glucopyranosido)-phenylacetic acid.

This substance was found abundantly in the oviduct of the mature female locust and white ootheca just after laying but hardly detected in immature female locust and coloured ootheca.

Résumé. J'ai démontré dans l'abdomen de la locuste femelle maturée, *Locusta migratoria*, l'existence d'une nouvelle substance diphénolique conjonctive. A l'aide de techniques chromatographiques sur papier, j'ai constaté que sa substance est identique à la 4-O-β-glucoside d'acide 3,4-dihydroxyphénylacétique.

S. TOMINO

Department of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo (Japan), December 17, 1962.

[4] H. D. GIBBS, J. biol. Chem. *72*, 649 (1927).

Submikroskopische Struktur von Vanadocyten. Ein Beitrag zur Vanadin-Anhäufung bei Tunicaten

HENZE[1] fand im Blut der Ascidie *Phallusia mamillata* Cuvier grünliche, maulbeerförmige Zellen, die sich durch zwei Besonderheiten auszeichnen: Schwarzfärbung mit Osmiumsäure und Farbumschlag des Indikators Methylrot von Gelb (pH > 6.3) nach Rot (pH < 3.2). Die Schwärzung beruht auf der Reduktion des OsO_4 durch das in den Zellen angereicherte 3wertige Vanadin; mit ihm zu einer hellgrünen Koordinationsverbindung verknüpfte Schwefelsäure[2] ist für die Indikatorreaktion verantwortlich. Dieser Blutzelltyp wurde von WEBB[3] Vanadocyten genannt und gegen die übrigen im Blut vorkommenden Zellarten[4–6] histomorphologisch abgegrenzt: «In freshly drawn blood it has a circular outline, though after standing some time, or in fixed material, it takes a morula form so that the outline becomes rosette-shaped. It is about 8 μ in diameter and contains a number (usually 8–10) of inclusions which almost entirely fill the cell, leaving only a small space in or near the centre for the nucleus, which is slung by bridles from a thin peripheral layer of cytoplasm».

Das elektronenmikroskopische Bild OsO_4-fixierter, geschnittener Vanadocyten von *Phallusia mamillata* (Figur 1) lässt sich gut mit dem lichtmikroskopischen vergleichen. Die Bezirke der Vanadinanhäufung, die wir *Vanadophoren* nennen, zeigen eine besonders hohe elektronenoptische Dichte. Sie nehmen im Schnitt eine Fläche von je 2–6 μ^2 ein und entsprechen den lichtmikroskopisch erkennbaren, hellgrünen Einschlüssen. Neben sehr dichten Vanadophoren kommen stets auch solche grösserer Durchlässigkeit für Elektronen vor. In diesen wird eine feinkörnige Innenstruktur erkennbar (Figur 2). Der unterschiedliche Grad der Dichte kann von der Schrumpfung der sehr empfindlichen Zellen oder auch vom Ausmass der Metallanhäufung herrühren. Dass die Absorption der Elektronen tatsächlich durch Vanadin und nicht durch Osmium bedingt ist, geht aus Vergleichspräparaten hervor, die nur mit Formalin fixiert wurden. Durch das Schneiden bedingte Stauchungs- und Vibrationseffekte weisen auf eine höhere Konsistenz der Vanadophoren, verglichen mit den übrigen Zellbestandteilen, hin. Die

[1] M. HENZE, Hoppe-Seyler's Z. *86*, 340 (1913).
[2] H.-J. BIELIG, E. BAYER, L. CALIFANO und L. WIRTH, Pubbl. Staz. Zool. Napoli *25*, 26 (1953).
[3] D. A. WEBB, J. exp. Biol. *16*, 499 (1939).
[4] W. C. GEORGE, Quart. J. microscop. Sci. *81*, 391 (1938/40).
[5] M. AZÉMA, Ann. Inst. océanographique *17*, 1 (1937/38).
[6] R. ENDEAN, Quart. J. microscop. Sci. *101*, 177 (1960).