this amine is thus about one third of the concentration in the brain. It is also seen that after the cross section the noradrenaline of the part caudal to the section disappears almost completely. This fact seems to us to indicate that the major part of the noradrenaline of the cord is located in nerve fibres, the cell bodies of which occur in an area of the central nervous system rostral to the section. This is taken as an additional proof for the view that the noradrenaline of the central nervous system is a neurohormone. Work is in progress in order to localize the cell bodies.

In contrast to other investigators, we have not been able to detect any significant amount of dopamine in the spinal cord of the rabbit or the rat. The values obtained by us were 0.01 µg/g or less, i.e. only about one hundredth of the amount found by McGEER and McGEER[4,5].

Zusammenfassung. Der Noradrenalingehalt im Rückenmark des Kaninchens beträgt 0.15 µg/g. Eine Querschnittsläsion durch das Rückenmark führt zu einer 90%igen Abnahme des Noradrenalingehaltes. Dies legt die Annahme nahe, dass Noradrenalin in Nervenzellen des Zentralnervensystems lokalisiert ist. Im Gegensatz zu anderen Untersuchern fanden wir im Rückenmark kein Dopamin.

T. MAGNUSSON and E. ROSENGREN

Department of Pharmacology, University of Gothenburg (Sweden), January 8, 1963.

[4] E. G. Mc GEER and P. L. Mc GEER, Canad. J. Biochem. Physiol. *40*, 1141 (1962).

[5] The work has been supported by grants from the Office of Aerospace Research, United States Air Force and the Swedish Medical Research Council.

Masking Action of Basic Proteins on Sialic Acid Carboxyls in Epithelial Mucins[1]

Histochemical studies on polysaccharides of different mucous-producing glands have clearly established that tissue basophilia, revealed with different cationic dyes (alcian blue, colloidal iron, azure A), is due to the presence of sialic acid (SPICER and WARREN[2], QUINTARELLI et al.[3]). Chemical investigations on salivary mucins further demonstrated that sialic acid occupies terminal positions and that mild acid hydrolysis releases sialic acid without further cleavage of the mucin molecule (GOTTSCHALK[4]).

Accordingly, when the mild hydrolytic treatment was applied to submaxillary gland sections it was found that in some animals basophilia was completely removed while in other animals similar results were obtained only when stronger acid solutions, higher temperatures and longer incubation times were applied (QUINTARELLI et al.[5]). Furthermore, the use of the specific enzyme neuraminidase demonstrated that whereas in some tissues sialic acid was easily split off from the glycoprotein molecule, in other animal glands neuraminidase had only a slight hydrolytic effect (QUINTARELLI et al.[5], WARREN and SPICER[6]). These findings suggested that, although most of the salivary mucins contained sialic acid, their structural configuration varied widely from one animal gland to another.

Recent chemical and histochemical studies on several mammal salivary mucins have disclosed that sialo-glycoproteins are present not only in the mucous-producing glands but also in the parotid (AURELI et al.[7]). Furthermore, treatment of parotid and submaxillary sections with proteolytic enzymes (pepsin, crude and crystalline papain) gave rise to opposite and quite unexpected results. While in some mammals proteolysis destroyed submaxillary mucins, thus leaving no substrate to be stained in the section, in other animal glands the staining for acid polysaccharides was greatly augmented after proteolytic treatment. These latter findings suggested that the sialic acid carboxyls responsible for the dye-binding were somewhat blocked by basic proteins (QUINTARELLI[8]), probably in the same manner as some acid mucopolysaccharide-protein complexes in the connective tissue ground substance (KELLY[9], FRENCH and BENDITT[10], QUINTARELLI[11]).

The results reported heretofore seemed to indicate that in some animal mucins sialic acid may be found in two different forms: an unbound form, susceptible to mild acid hydrolysis, and a bound one which could only be revealed after the action of proteolytic enzymes. Since these findings needed further examination, additional histochemical procedures were used in an effort to assess more data which could either substantiate or contradict the original results.

Submaxillary glands of monkeys (*Macaca mulatta*) were utilized together with dog parotids. Fresh tissues were fixed in alcohol-formaldehyde, dehydrated, cleared and embedded in the usual fashion. Deparaffinized sections were immersed in a 0.1 *N* Na-acetate/HCl solution, pH 2.5 at 75° for 1, $1^1/_2$ and 2 h. Control sections were incubated in distilled water at the same temperature and for the same lengths of time. After treatment, sections were rinsed in distilled water, dried and then incubated in pepsin or crude papain for 2 h. Papain was activated by incubating the enzyme at 37° for 30 min in 20 ml of 0.02 *M* acetate buffer, pH 5.4, which contained KCN and E.D.T.A. in a concentration of 5 µm. Pepsin was prepared in a 0.02 *N* Na-acetate/HCl solution, pH 2.5 containing

[1] This work was supported by research grants (D-1325 and D-1326) from the National Institutes of Health, United States Public Health Service.

[2] S. S. SPICER and L. WARREN, J. Histochem. Cytochem. *8*, 135 (1960).

[3] G. QUINTARELLI, S. TSUIKI, Y. HASHIMOTO, and W. PIGMAN, Biochem. biophys. Res. Comm. *2*, 423 (1960).

[4] A. GOTTSCHALK, Biochem. biophys. Acta *24*, 649 (1957); *Ciba Foundation Symposium on the Chemistry and Biology of Mucopolysaccharides* (Little, Brown & Co., Boston 1958).

[5] G. QUINTARELLI, S. TSUIKI, Y. HASHIMOTO, and W. PIGMAN, J. Histochem. Cytochem. *9*, 176 (1961).

[6] L. WARREN and S. S. SPICER, J. Histochem. Cytochem. *9*, 400 (1961).

[7] G. AURELI, M. RIZZOTTI, G. FERRI, and A. A. CASTELLANI, Arch. Ital. Biol. Or. *2*, 52 (1962).

[8] G. QUINTARELLI, Ann. N.Y. Acad. Sci., in press (1963).

[9] J. W. KELLY, Arch. Biochem. *55*, 130 (1955).

[10] J. E. FRENCH and E. P. BENDITT, J. Histochem. Cytochem. *1*, 321 (1953).

[11] G. QUINTARELLI, Arch. Or. Biol. *2*, 277 (1960).

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

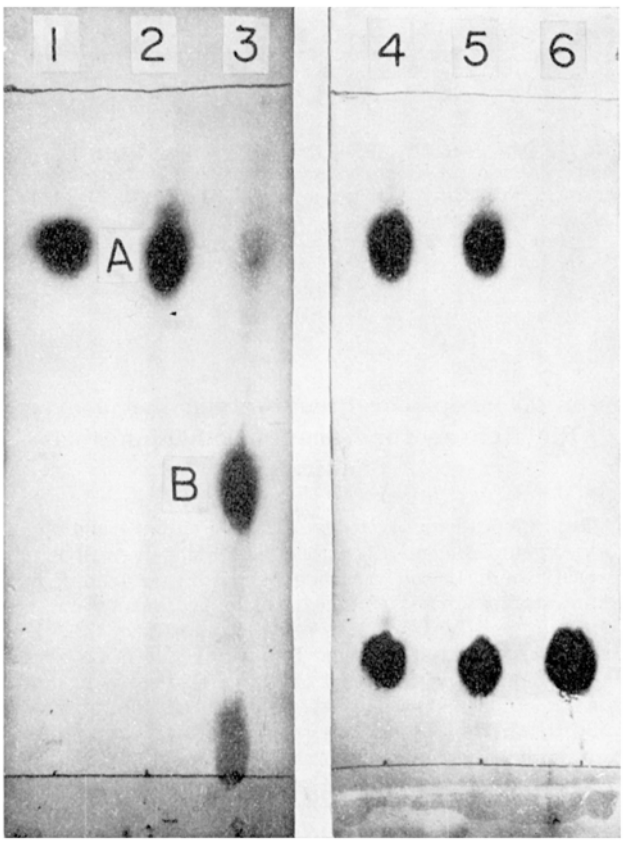

Fig. 1. Paper chromatograms of phenol derivatives found in the mature female locust, and its hydrolysates.—Ascending method was used. The solvent was a mixture of *n*-butanol, acetic acid and water (4:1:2 by volume). Filter paper was Toyo No. 51. Colour was developed with Folin-Ciocalteu reagent (lefthand 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).