number of reticulocytes was observed. The increase failed to appear in persons subjected to the same amount of strain, but without spirometrically demonstrated oxygen deficiency. Similarly, the increase failed to appear in persons with impaired pulmonary function if the inspired air contained 60 per cent of oxygen during the course of the test.

Identification of Enteramine and Enteramine-Related Substances in Extracts of Posterior Salivary Glands of *Octopus vulgaris* by Paper Chromatography

Concentrated acetone extracts of posterior salivary glands of *Octopus vulgaris* were chromatographed on paper. Hereby we succeeded in localizing in well-defined spots enteramine A, enteramine I, and some other enteramine-related substances.

The identification of the enteraminic spots was accomplished by means of a whole series of colour reactions and by specific biological reactions.

The extracts submitted to chromatography had concentrations corresponding, per cc., to 2–20 g fresh salivary tissue. They were applied on Whatman No. 1 or No. 4 paper, in amounts of 0·002–0·01 cc.

Among the solvents with which we experimented, butanol saturated with HCl n gave the best results in unidimensional chromatography. The acid solvent was followed, in the bidimensional chromatography, by the alkaline mixture pyridine–amylalcohol–water (2:2:1).

By spraying the chromatograms with a solution of the diazonium salts of paranitroaniline or sulfanilic acid and exposing them to ammonia vapours, numerous spots giving a positive alkaline azoreaction could be developed. They are schematically illustrated in Fig. 1 and 2. The

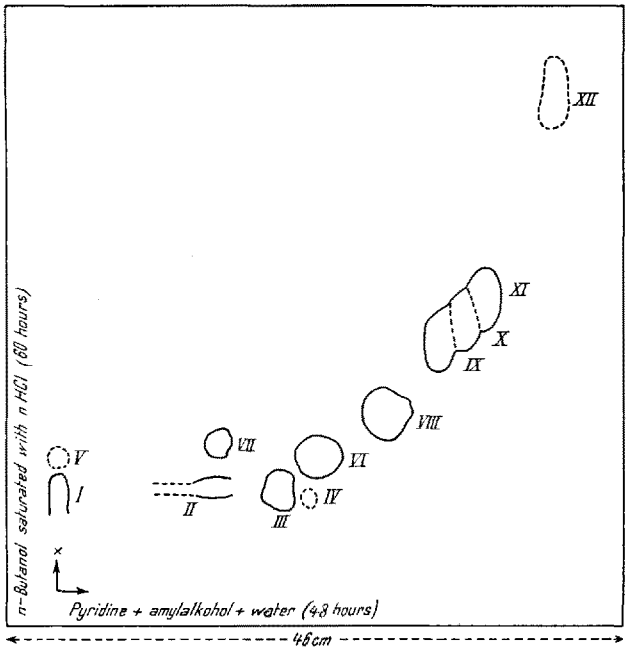

Fig. 1. – At the mark × ·01 cc. of the summer extract Vulg. CR_1, corresponding to ·2 g fresh salivary tissue.

first refers to a salivary extract obtained in summer, 6 months old, and the second to a winter extract, quite recent and prepared from absolutely fresh material (standard extract).

The problem of the nature of the other spots will be discussed in a future communication; at the present we permit ourselves to affirm that spots III and IX surely contain enteramine, as demonstrated by their peculiar chemical and biological characteristics. Spot III corresponds to enteramine A, spot IX to enteramine I[1]. We are inclined to admit that enteramine I may be, at least partially, the product of an initial change of enteramine A: this view is supported by the usual weakness of spot IX in the chromatograms obtained with our fresher extracts.

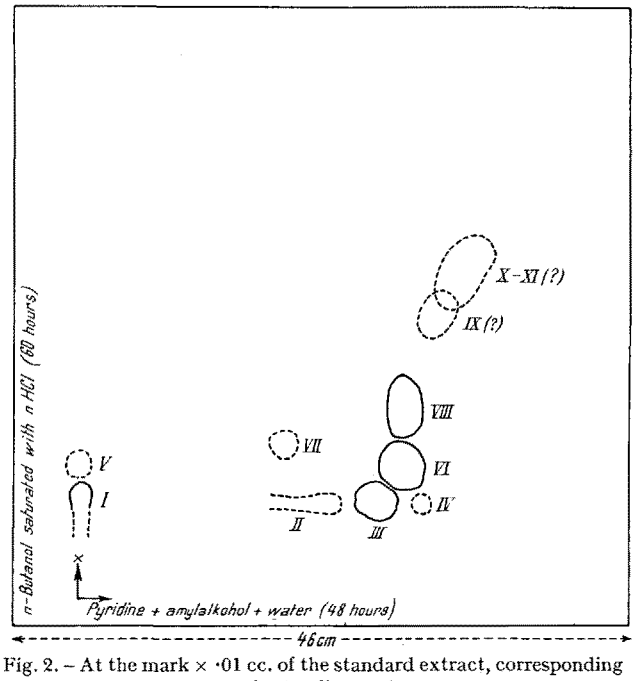

Fig. 2. – At the mark × ·01 cc. of the standard extract, corresponding to ·2 g fresh salivary tissue.

The enteraminic or enteramine-like nature of spots I, II, IV is probable, but as yet not certain: in fact, they give all, or nearly all, the characteristic colour reactions of enteramine, from which they are, in this regard, practically indistinguishable, but they do not show the peculiar biological actions of enteramine. It is possible that the substances constituting spots I-II-IV preexist in the fresh tissue, but it is equally possible that they simply represent some decomposition products of enteramine.

The following approximative Rf values were obtained for the three enteraminic spots which separate better on unidimensional chromatograms: II = 0·11; III = 0·20; IX = 0·54.

Here are now, briefly, the data on which the above-mentioned conclusions are based:–

Colour reactions of the enteraminic spots. On untreated chromatograms, on which the solvent has just dried, spot III usually appears as a weak yellowish shade, which in time tends to become accentuated and to darken. Spot I, likewise, is often well visible, from the beginning as a reddish-yellow shade. Later on, spot IX and, much less distinctly, spots II and IV are delineated.

The true colour reactions of the enteraminic spots are, however, the following:–

[1] V. ERSPAMER, Naunyn-Schmiedebergs Arch. *200*, 60 (1942); Acta pharmacol. *4*, 213 (1948).

(a) Coupling reaction with the diazonium salts of paranitroaniline or sulfanilic acid. Already in an acid medium, this yields a peach-red coloration (more intensive with paranitroaniline), which appears gradually, but is very persistent (*acid azoreaction*). The colour turns to fuchsin-violet (paranitroaniline) or to brownish-violet (sulfanilic acid) upon exposing the wet chromatograms to ammonia vapours (*alkaline azoreaction*).

(b) Yellowish reaction, up to orange, with sodium nitrite + HCl. By consequent treating of the chromatograms with N-ethyl-α-naphthylamine hydrobromide, the original colour of the spots is not modified.

(c) Brownish, violet, or reddish reaction with potassium bichromate, sodium persulfate, hydrogen peroxide, potassium iodate, mercuric acetate (or mercuric bichloride + sodium acetate), ferric chloride, potassium ferricyanide.

(d) Immediate brown-blackish reaction with ammoniacal silver nitrate, still appreciable with the extract obtained from 0·15 mg fresh tissue. Immersing and washing the chromatograms in 5% sodium thiosulfate markedly decreases the intensity of the silver reaction.

(e) Blue reaction, turning to violet and to bottle-green, with an alcoholic acid solution of p-dimethylaminobenzaldehyde.

(f) Immediate blue reaction with the Folin-Ciocalteu reagent (*acid Folin reaction*), which is markedly accentuated by aftertreating the chromatograms with sodium carbonate or with ammonia vapours (*alkaline Folin reaction*).

(g) Blue reaction, upon heating, with the sulfomolybdic reagent.

(h) Bluish-violet or brownish-violet reaction with the Gerngros-Voss-Herfeld reagent (1% alcoholic solution of α-nitroso-β-naphthol + nitric acid).

(i) Brownish-violet, brownish or yellowish reaction with nitric acid (xanthoproteic reaction).

(j) Olive-green reaction with the Millon reagent.

(k) Pink reaction, turning to brownish, by ultraviolet irradiation of the wet chromatograms.

(l) Yellow fluorescence reaction in Wood's light, which begins 15–25 minutes after spraying the chromatograms with 8–12% NaOH, intensifies later and persists, slowly weakening, for days and weeks.

In visible light the alkali-treated enteraminic spots appear at first pink, then brownish.

(m) Yellow reaction with ninhydrin, which progressively accentuates. The reaction is negative for spot IX, doubtful for spots I–II–IV.

The enteraminic spots do not give any colour reaction with the Quastel reagent nor with the Visher and Chargaff reagent for purine and pyrimidine derivatives.

The acid azoreaction, all reactions dealt with in paragraphs b, c, d, j, and k, as well as the acid Folin reaction, the sulfomolybdic reaction and the blue reaction with p-dimethylaminobenzaldehyde are exclusively given, on the paper chromatograms of the salivary extracts, by the enteraminic spots.

Spot III is far the most reactive, followed by spots IX and I, then by spot II and lastly by spot IV.

It is reasonable to suppose that the colour tonalities obtained, corresponding to the enteraminic spots, with the various afore-mentioned reactions, should be alike or closely related to those which would be given *in vitro* by pure enteramine.

Biological activity of the eluates of the enteraminic spots. The aqueous eluate of spot III possesses, *per se*, an immediate powerful stimulating effect on the atropinized œstrus-uterus of rats and mice, the duodenum of rats, the urinary bladder of dogs[1], and the heart of Mollusca[2].

The eluate of spot IX, when obtained with distilled water, *per se*, is quite inactive, but acquires a strong stimulating action on the above-mentioned tests when alkalinized and then briefly heated, or when obtained with a phosphate buffer at p_H 7·2–7·8 instead of distilled water.

Up to now we have not succeeded in demonstrating any peculiar enteraminic activity for the eluates of spots I-II-IV, which perhaps is due to their low content of specific substances.

The eluates of all non-enteraminic spots, as evidenced by the alkaline azoreaction, are quite ineffective on the biological tests of enteramine, or they display a weak depressive action.

These first investigations (a fuller account will appear elsewhere) were completely confirmed by similar researches conducted on extracts of posterior salivary glands of *Octopus macropus* and *Eledone moschata*, extracts of the hypobranchial body of *Murex trunculus* and *Murex brandaris*, as well as extracts of mammalian spleen and intestinal mucosa.

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Pharmacological Institute, University of Bari, and Farmitalia S.A. Research Laboratories, Milan, April 5, 1950.

Zusammenfassung

Konzentrierte Extrakte der hinteren Speicheldrüsen von *Octopus vulgaris* wurden auf Papier chromatographiert. Zahlreiche Flecke konnten durch chemische und teilweise auch durch biologische Reaktionen differenziert werden. Hievon waren zwei sicher enteraminhaltig (Fleck III = Enteramin A; Fleck IX = Enteramin I). Drei andere enthalten wahrscheinlich enteraminähnliche Substanzen (Flecke I, II, IV). Die an mehr als 20 Farbreaktionen erhobenen Befunde werden kurz erörtert.

Antihistaminic Effect of Eosinophil Leukocytes

A possible theory of the pathological correlation between allergic states and increased number of the eosinopil leukocytes is yet lacking. Our experiments regarding the role of eosinophils in normal and pathologic conditions were based on the following theoretical considerations:–

(1) Reports of Samter[1] and Ayres[2], seem to prove that the so-called Charcot-Leyden crystals have their origin in the eosinophil leukocytes. (2) According to Wrede[3] spermine (one of the biogenic amines) is an important component of the above-mentioned crystals.

Utilizing the results of Ackermann and Wasmuth[4], who found an antihistaminic activity of spermine on isolated guinea-pig ileum, we investigated its antihistaminic property in several other animal experiments reported below.

If we can obtain sufficient data to demonstrate such an antihistaminic activity, and if we accept the cited results of the literature, one can suggest that the eosinophils contain a substance with antihistaminic property.

After having demonstrated a real antihistaminic activity of spermine, we made further experiments using

[1] V. Erspamer, Naunyn-Schmiedebergs Arch. *196*, 343 (1940) and unpublished data.

[2] V. Erspamer and F. Ghiretti, to be published.

[1] M. Samter, J. Allergy *18*, 221 (1947).

[2] W. W. Ayres, Blood *4*, 595 (1949).

[3] F. Wrede, F. Boldt, and E. Buch, Z. physiol. Chem. *165*, 155 (1927).

[4] D. Ackermann and W. Wasmuth, Z. physiol. Chem. *259*, 28 (1939).