Using as a guide the coupling reaction, numerous other colour reactions were carried out with the purpose of better characterizing the spots put in evidence and of more closely defining their chemical nature. The procedures for these reactions will be taken up in detail in another paper.

The results obtained with the *Vulgaris*-II extract (and for spot XIII with the *Macropus* standard extract) are given in Tables II and III.

The simultaneous positivness of the coupling reaction in an alkaline medium, of the Folin-Ciocalteu reaction and of the Gerngros-Voss-Herfeld reaction allows us to attribute a phenolic nature to the twelve spots I–XII; here the term "phenolic" should be understood in a broad sense.

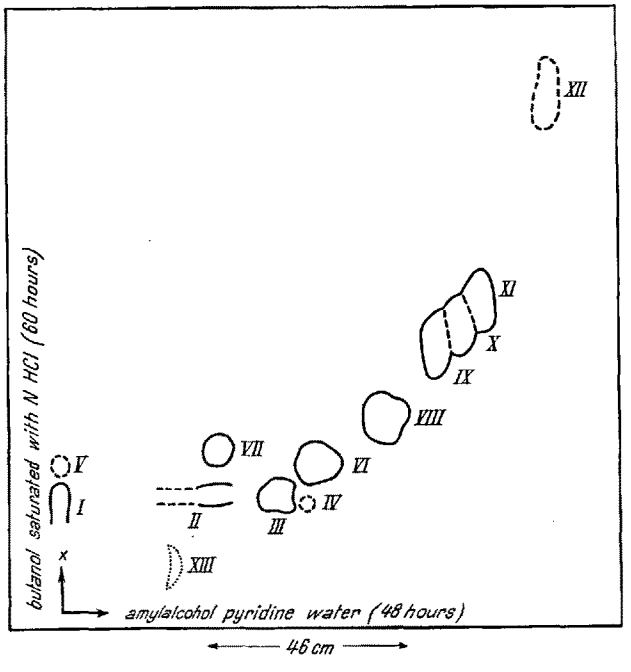

Fig. 2. – At the mark x, 0·01 cm³ of the *Vulgaris*-II extract, corresponding to 0·2 g fresh salivary tissue.

For spots I–X this view is makedly strengthened by the positivness of the Millon reaction; for spots I, II, III, IV, and IX, also by the positivness of the silver reaction and of the oxydation reactions.

The positivness of the coupling reaction in an acid medium and of the *p*-dimethyl-aminobenzaldehyde reaction are strongly indicative for the presence of an indolic nucleus in the substances constituting spots I, II, III, IV, and IX.

Numerous monodimensional chromatograms were then extracted with distilled water or with saline and the eluates of the individual Pauly-positive spots biologically tested, yielding the following results:

(a) Both spots II and IX demonstrate a potent enteraminic action (oestrus-uterus and duodenum of the rat, urinary bladder of the dog), the first spot immediately, the second only after a congruent treatment in an alkaline medium. Their identity with enteramine A and I is beyond question[1].

(b) After ultraviolet irradiation, spot VII shows an intense adrenaline-like action on the blood pressure and on various isolated organs. It surely contains octopamine[2].

(c) Spot VIII displays a tyramine-like pressor action. Exact superimposition of this spot and tyramine control spots, even in bidimensional chromatograms, sufficiently confirms its presumed tyraminic nature.

(d) Spot XIII, particularly evident on the chromatograms of *Octopus macropus*, stimulates the atropinized small intestine of the guinea pig, and the stimulating action is completely inhibited by synthetic antihistamines. Hence, it consists of histamine.

(e) So far, there have been found no characteristic biological reactions for the other spots. It is, however, highly probable that spot VII results from tyrosine (as shown by its exact superimposition on tyrosine control spots and by the parallel displacement of spot VII and of tyrosine control spots by changing the solvent), and it is probable that spots I, II, and IV are of an enteraminic nature. According to the characteristics of many colour reactions, spot X may be considered octopamine-like, and spot XI tyramine-like.

Very likely the constituents of spots III, VI, VIII, and XIII are already present in the fresh salivary tissue. The preexistence of the constituents of spots I, II, and IV is doubtful, improbable that of the constituents of the other Pauly-positive spots, which reasonably are to be considered as products of tissue autolysis (spot VII) or as alteration-products of enteramine, octopamine and tyramine (spots IX, X, XI).

It appears easily evident from table I that the Pauly-positive substances contained in the salivary extracts conspicuously vary from species to species. Any generalisation of data obtained in one or even in more species of *Octopoda* is therefore quite arbitrary.

V. Erspamer and G. Boretti

Pharmacological Institute, University of Bari, and Farmitalia S.A., Research Laboratories, Milan, May 25, 1950.

Zusammenfassung

Dreizehn verschiedene, mit Diazoniumsalzen kupplungsfähige Substanzen wurden mittels Papierchromatographie in Azetonextrakten der hinteren Speicheldrüsen von *Octopoda* identifiziert und getrennt.

Auf Grund der durch die Anwendung mehrerer Farbreaktionen erhaltenen Resultate sind zwölf solcher Substanzen als von phenolischer oder indolischer Natur zu betrachten (darunter besonders wichtig A-Enteramin, I-Enteramin, Octopamin und Tyramin); die dreizehnte kupplungsfähige Substanz ist als Histamin zu identifizieren.

Extrakte der drei verschiedenen hier in Betracht gezogenen *Octopus*-Arten haben ganz unähnliche Chromatogramme geliefert.

[1] V. Erspamer, Naunyn-Schmiedebergs Arch. *200*, 60 (1942); Acta pharmacol. *4*, 213 (1948). – V. Erspamer and G. Boretti, Exper. *6*, 428 (1950).

[2] V. Erspamer, Acta pharmacol. *4*, 224 (1948).

Purified Leukocyte Suspensions with Antihistaminic Activity

Our previous investigations suggested that human (eosinophil) leukocytes are capable of exerting antihistaminic effect on guinea-pigs. In these experiments[1] leukocyte suspensions were examined as isolated by the Szilard-method[2]. The present communication deals with the purification and biological testing of purified suspensions.

[1] A. Kovács, Exper. *6*, 349 (1950).

[2] P. Szilard, Pflügers Arch. ges. Physiol. *211*, 597 (1926).

Suspensions were rubbed by quarz sand. This procedure was followed by a three-fold extraction with equal amounts of ether. The ether was evaporated and absolute ether than added. The dried material (1–2 mg per 50–100 cm^3 human blood sample) was investigated after a distillation process.

Histamine aerosol[1] and Magnus experiment were our choice for testing the purified material on guinea-pigs. This material is protein-free and does not contain histamine. It, however, possesses the greater part of the antihistaminic activity.

Before the injections the material (1–2 mg dry weight) was dissolved in 2–3 cm^3 ether, and 0,2 cm^3 of Ol. Helianthi with 1 cm^3 of distilled water were added. The ether was evaporated before using.

Tests were performed in 0·4 per cent histamine aerosol 5–6 hours after the intraperitoneal injections. The table represents our results. As is shown, the purified material preserved its activity, which ran parallel with the per cent distribution of the eosinophils.

The experiments on isolated guinea-pig ileum gave similar results. The histamine-induced spastic contraction can be prevented with this purified material. The optimum time for adding histamine, however, is 30–40 minutes after the inhibiting material was administered.

The role of eosinophils in the effect of purified leukocyte suspensions on guinea-pigs closed in histamine-aerosol.

Number of experiments	Total leukocyte count of suspension in million	Total eosinophil count of suspension in million	Exposure time in 0·4 per cent histamine spray: above 10 minutes	above 6 minutes	below 6 minutes
10	250–850	30–150	7	3	–
7	250–450	15– 30	1	1	5
10	250–600	5– 15	–	1	9

Finally, using large amounts of cattle-blood as initial material[1], we succeeded in obtaining crystallization, and we have found that 10–15 micrograms of the repeatedly recrystallized material (also containing the lipids) inhibit the histamine-induced contraction of the guinea-pig ileum even after 5 minutes.

Further experiments are in progress.

A. KOVÁCS and É. JUHÁSZ

Department of Pharmacology University of Szeged, Hungary, October 23, 1950.

Zusammenfassung

In einer früheren Untersuchung konnte wahrscheinlich gemacht werden, daß die eosinophilen Leukozyten einen Stoff enthalten, der als Antihistaminikum wirkt.

Es gelang jetzt, diese Substanz weitgehend zu reinigen. Der eiweiß- und histaminfreie Stoff verhindert den Histamineffekt am isolierten Meerschweinchendarm und ebenso die durch Histamin-Aerosol ausgelösten Krämpfe der Bronchialmuskulatur.

[1] The quarz-rubbed suspensions are filtered off on commercial filter paper. Extractions, etc. (see above) with ether are rapidly followed by filtrations. The evaporation is carried out at room temperature, and the procedure is repeated 2- to 3-fold. Thereafter we dissolve material in absolute ether and dry it at room temperature. At this stage we have homogeneous crystals, which, however, contain some oil-like material, too. Further purification is in progress.

Electrophoretic Measurements of Human Spermia

The present paper is a preliminary report on the comparison of the electrophoretic mobilities of normal and pathological human spermia. It was thought that pathological conditions may influence the surface potential of the cells so that some correlation might be found between clinical and electrophoretic mobility.

In the following experiments each ejaculate was examined by the method described by one of us[1]. The material described as "normal" originated from men of proven fertility, the abnormal samples from men whose semen was found to be pathological in tests at different periods.

The results of some measurements at pH–7,8 are summarized in the following table.

No. of sample	No. of sperms per cm^3	Clinical description	Mobility $\frac{cm^2}{sec/Volt}$
32	90,625,000	Normospermia	$6{\cdot}1\ 10^{-5}$
48	1,562,500	Hypozoospermia	$7{\cdot}8\ 10^{-5}$
50a	60,000,000	Normospermia	$8{\cdot}6\ 10^{-5}$
53	6,250,000	Hypozoospermia	$8{\cdot}7\ 10^{-5}$
58	60,000,000	Normospermia	$8{\cdot}0\ 10^{-5}$

No significant difference in mobility of sperms between normal and pathological semen could be detected. This result parallels the work[2] done on seminal plasma in which no difference was observed between electrophoretic patterns of normal and pathological samples.

Some discussion is in order on the influence of the velocity of sedimentation, of diffusion and of orientation on the significance of the results.

It might be argued that the sedimentation of the cells is sufficiently rapid to cause an apparent increase in the velocity of the descending boundary and a corresponding decrease in the ascending boundary velocity. Observations on the velocity of sedimentation in dilute suspension show this factor to be insignificant. In fact, the velocities of both ascending and descending boundaries were equal within the limits of the experimental error.

A similar objection might be raised concerning the observed orientation of the cells in the electric field. It is known from previous experiments[3] that the spermia tend to orient tail forward in the direction of the field. It could, therefore, be argued that the observed velocity is actually the difference between the electric drag of the cells and their natural movement in the opposite direction. Our microscopic observations proved that an appreciable orientation of the motile cells occurs only at higher field intensities. At 2·5 V/cm, no orientation of the motile cells could be observed; on the other hand, immobile cells orient easily and rapidly. We may, therefore, safely assume that no directed movement opposite to the direction of the electrophoretic flow was operative under the conditions of the experiments.

[1] C. A. JOËL, *Studien am menschlichen Sperma* (B. Schwabe & Co., Basel 1942).

[2] S. GRAY and C. HUGGINS, Proc. Soc. Exp. Biol. Med. *50*, 2 (1942). – V. ROSE, H. MOORE, and H. SIKOVSKI, Proc. Sec. Exp. Biol. Med. *54*, 179 (1943).

[3] R. A. ABRAMSON, *Elektrokinetic Phenomena and their Application to Biology and Medicine*, p. 274 (New York, 1934).