(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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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).

Effect of human leukocyte suspensions on guinea-pigs placed in histamine spray 5 to 7 hours after intraperitoneal injection.

Number of experiments	Total leukocyte count of suspensions in millions	Total eosinophilic count of suspensions in millions	Time in 0·4 per cent spray above 10 minutes	above 6 minutes	below 6 minutes
15	300–600	15–50	13	1	1
10	250–600	2–12	1	2	7

several leukocyte-suspensions. If such suspensions have any activity, according to the above suggestions a continous correlation should exist between the activity and the total number of eosinophils present in the suspensions used.

Methods

(1) *Histamine-spray test.* Guinea-pigs of 400–500 g body weight were placed in a closed area (c. 2 l). A 0·4 per-cent histamine solution was nebulized in it continuously. A lethal bronchospastic attack develops normally within 2 to 5 minutes. We count a real *protective effect* if the animal survives 10 minutes in the spray. During this, the animal could inhale 2–3 times the amount of the lethal dose.

(2) *Chemosis test of* JANSCÓ. One drop of an 1·5–2·0 per-cent solution of histamine instillated into the eyes of guinea-pigs with weight as above causes a marked chemosis within 10 minutes. Known antihistaminic drugs given previously abolish this effect.

(3) JANSCÓ's *India ink test.* India ink stabilized with gelatine is given intravenously to normal adult mice. The circulating time of India ink is measured by photometerizing filter papers with successive drops of mouse blood by means of a photocell. Synthetic antihistaminics increase the circulating-time about twice.

(4) *Anaphylactic shock of guinea-pigs.* Sensibilization with 0·5 ml of human serum intraperitoneally. Reinjection after 20–22 days, using 0·2 ml, intracardially. A sign of protection is the prolongation of the survival time, the duration of which seems to be suitable for some quantitative determinations too.

Results

I. *Experiments with spermine phosphate.* Spermine phosphate is acidified slightly to become soluble if heated. Three intraperitoneal injections were given to guinea-pigs on the 1, 3 and 5th days, 50 mg for each injection. 15 animals were tested. After the third injection the protection begins at 3·5 to 4·5 hours for histamine spray and at 7 to 9 hours as regards the chemosis test with a duration of 7 to 16 hours for the former and 10 to 30 hours for the later[1].

The prolongation of circulation time of India ink in 15 mice given previously 0·5 mg spermine phosphate subcutaneously agrees very well with results obtained with synthetic antihistaminics. The sudden death following reinjection in anaphylactic experiments could not be observed in any of the cases. 12 animals survived 1·5 to 2 hours and 3 remained alive.

II. *Experiments with leukocyte suspensions.* The first experiments were made using leukocyte suspensions of rabbits prepared by the SZILÁRD method[2]. 10 suspension in which the total leukocyte count ranged from 300 to 400 millions, with a total number of eosinophils and pseudo eosinophils of 50–100 millions, were given to guinea-pigs in the amount of 1 to 2 ml intraperitoneally. These animals showed a remarkable protection against histamine spray as well as in the chemosis test.

[1] In several animals a remarkable loss of weight arose during the spermine administration.

[2] P. SZILÁRD, Pflügers Arch. *211*, 597 (1926).

The following experiments now in progress were carried out with leukocyte suspensions of human subjects with diverse diseases. After rubbing suspensions the injections were given to guinea-pigs intraperitoneally in the amount of 1 to 2 ml. The results obtained after 5 to 7 hours are represented on the Table.

Conclusions

(1) It was suggested that eosinophil leukocytes contain a substance with antihistaminic activity. Spermine, a biogenic amine, according to WREDE *et al.* present in Charcot-Leyden crystals, which are derived from eosinophil leukocytes according to our present knowledge, was tested in several antihistaminic experiments in animals. Remarkable antihistaminic activity was demonstrated in four tests.

(2) Leukocyte suspensions prepared from rabbit blood and given to guinea-pigs also exert a marked antihistaminic activity in histamine spray. This effect seemed to stand in linear relation with total eosinophil count.

(3) Similar experiments made by using leukocyte suspensions of human beings with various diseases confirmed the above suggestion.

It seems possible to us that one of the functions of eosinophils may be participation in the neutralization of histamine liberated by allergic attacks. Further experiments are needed to prove this hypothesis.

A. KOVÁCS

Pharmacological Institute, University of Szeged, Hungary, February 2, 1950.

Zusammenfassung

1. Nach Ansicht des Verfassers enthalten die eosinophilen Leukozyten eine antihistaminartig wirkende Substanz. Das Spermin ist nach WREDE ein Bestandteil der Charcot-Leydenschen Kristalle, die nach dem heutigen Stand der Kenntnisse aus den Eosinophilen entstehen. Spermin zeigte in verschiedenen Testversuchen am Tier eine ausgesprochene Wirkung gegen Histamin.

2. Mit intraperitonealer Injektion von Leukozytensuspensionen aus Kaninchenblut ließen sich Meerschweinchen gegen die Effekte eines Histamin-Sprays schützen. Die Wirkung ist proportional der Zahl der eosinophilen Zellen.

3. Ähnliche Versuche wurden mit Leukozytensuspensionen aus dem Blut von Menschen, die eine Eosinophilie aus verschiedenen Ursachen zeigten, unternommen. Dabei wurden vollkommen übereinstimmende Resultate erhalten. Es darf angenommen werden, daß die eosinophilen Leukozyten im Organismus eine neutralisierende Wirkung gegenüber dem bei allergischen Reaktionen frei werdenden Histamin ausüben.