

une véritable fonte de noyaux du parenchyme splénique. Fait essentiel, la réduction de l'A.P.N. et de l'A.D.N. est parfaitement réversible.

Il apparaît ainsi une nouvelle caractéristique biochimique de la labilité du parenchyme splénique.

M. JACOB, L. MANDEL et P. MANDEL

Institut de chimie biologique, Faculté de médecine, Université de Strasbourg, le 4 février 1951.

Summary

After a prolonged protein-free diet, the spleen loses an average of 50 per 100 of its pentosenucleic acid and up to 55 per 100 of its desoxypentosenucleic acid.

The loss of desoxypentosenucleic acid reveals a real disappearance of the cell nuclei of the spleen's tissue. In this respect the spleen differs from other organs in which desoxypentosenucleic acid remains unchanged in these conditions.

The loss of pentosenucleic acid and desoxypentose-nucleic acid of the spleen is a completely reversible process.

Substances of a Phenolic and Indolic Nature Present in Acetone Extracts of the Posterior Salivary Glands of Octopoda (*Octopus vulgaris*, *Octopus macropus* and *Eledone moschata*)

They were individualized, separated, and characterized with the help of paper partition chromatography.

After numerous attempts with various solvents, butanol saturated with N HCl, and, to a lesser degree, butanol saturated with water + 10% acetic acid were found to be best suited in the monodimensional chromatography. These solvents were followed, in the bidimensional chromatography, by the mixture amyl alcohol-pyridine-water (2:2:1). Whatman No. 1 paper was used.

Of the various acetonic extracts taken into consideration in our experiments, some were prepared from very fresh material removed directly after the animals were killed (standard extracts), others were obtained from somewhat older material.

The solvent was driven off at diminished pressure, and the remaining aqueous liquids were extracted with petroleum ether for removal of fats and then reduced to the desired concentration. In general, aqueous liquids corresponding, per cm³, to 10–20 g fresh salivary tissue were used. They were put on the paper in amounts of 0.002–0.02 cm³.

All runs have been for 48–60 hours, at room temperature. After drying of the paper sheets, the spots of a presumable phenolic or indolic nature were detected by means of various colour reactions, more or less specific for the phenols and the indols themselves.

Leaving aside the ninhydrin test, of small importance in the present researches, the reaction which gives positive results at the level of the major number of spots is the

coupling reaction, in an alkaline medium, with the diazonium salts of paranitroaniline or sulphanilic acid.

The immediate colour tonalities of the coupling spots, often very different from the definitive ones, can be reproduced as many times as desired by exposure of the dry papers to ammonia vapours.

After alkalizing with non volatile bases the immediate colour tonalities remain unchanged.

Figures 1 and 2 schematically represent two chromatograms, one a mono- and the other a bidimensional chromatogram, obtained with the *Vulgaris*-II extract and developed by the diazonium salt of sulphanilic acid (Pauly's reagent).

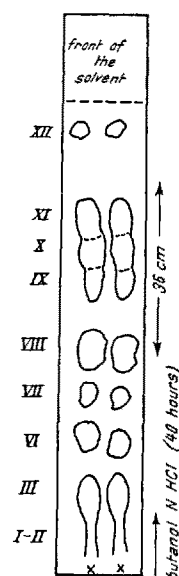


Fig. 1.—At the marks x, 0.002 cm³ of the *Vulgaris*-II extract, corresponding to 0.04 g fresh salivary tissue.

The Pauly reaction thus puts in evidence 12 spots, which clearly separate, with the exception of group IX-X-XI. On the chromatograms of *Octopus macropus* and of *Eledone moschata* there appears an additional thirteenth spot (outlined by dots in figure 2).

Table I informs about the presence or non-presence, on the chromatograms obtained with our different extracts, of the individual Pauly-positive spots and also reveals the intensity of the colour reaction.

The following approximative *R_f* values were obtained on monodimensional *Vulgaris*-II chromatograms (butanol saturated with N HCl):

II = 0.11; III = 0.20; VI = 0.27; VII = 0.37; VIII = 0.45; IX = 0.54; X = 0.60; XI = 0.68; XII = 0.97.

Table I

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Vulgaris-standard extract	+	(+)	+++	(+)	(+)	+++	(+)	+++	—	—	(+)	—	?
Vulgaris-I extract . . .	+	(+)	+++	(+)	(+)	+++	++	+++	+	+	+	+	?
Vulgaris-II extract . . .	+	(+)	+++	(+)	(+)	+++	++	+++	+	+	+	+	?
Vulgaris-III extract . . .	+	(+)	++	(+)	(+)	+++	(+)	+++	?	?	+	(+)	?
Vulgaris-IV extract . . .	+	(+)	++	(+)	(+)	+++	+	+++	(+)	(+)	+	(+)	?
Macropus-standard extract	—	—	—	—	—	(+)	(+)	++++	—	—	+	—	+
Eledone-standard extract.	+	+(+)	++++	?	?	+	(+)	?	+	—	—	—	+

Table II

	I	II	III	IV	IX	V	VI	VII	VIII	X	XI	XII	XIII
Coupling reaction with the diazonium salt of <i>p</i> -nitroaniline													
(a) Acid medium	+	(+)	++	(+)	(+)	-	-	-	-	-	-	-	-
(b) Alkaline medium	+	(+)	+++	(+)	+	(+)	+++	+++	+++	+	+	+	+
Pauly reaction													
(a) Acid medium	(+)	(+)	+	(+)	(+)	-	-	-	-	-	-	-	-
(b) Alkaline medium	+	(+)	+++	(+)	+	(+)	+++	+++	+++	+	+	+	+
Folin-Ciocalteu reaction													
(a) Acid medium	(+)	(+)	++	?	(+)	-	-	-	-	-	-	-	-
(b) Alkaline medium	+	+	+++	?	+	?	+++	+++	+++	(+)	(+)	(+)	(+)
Gerngros-Voss-Herfeld reaction	(+)	(+)	++	?	(+)	(+)	++	++	++	+	?	+	+
Millon reaction	(+)	(+)	+	?	(+)	(+)	++	++	++	+	?	+	+

¹ Immediate and ² definitive colour shades after alkalinizing with ammonia.

Table III

	I	II	III	IV	IX	V	VI	VII	VIII	X	XI	XII	XIII
<i>p</i> -Dimethylamino-benzaldehyde	+	(+)	++	?	(+)	-	(+)?	-	-	-	-	(+)	-
Ammoniacal silver nitrate	+	+	+++	?	+	-	-	-	-	-	-	-	-
Potassium iodate, potassium ferricyanide, potassium bichromate, ferric chloride, u. v. irradiation, etc.	(+)	(+)	+	?	(+)	-	-	-	-	-	-	-	-
Fluorescence in Wood's light	(+)	(+)	++	?	+	-	-	-	-	-	-	-	-
Ninhydrin	?	(+)	++	?	?	?	+	+	+	?	?	-	+

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 positiveness 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.

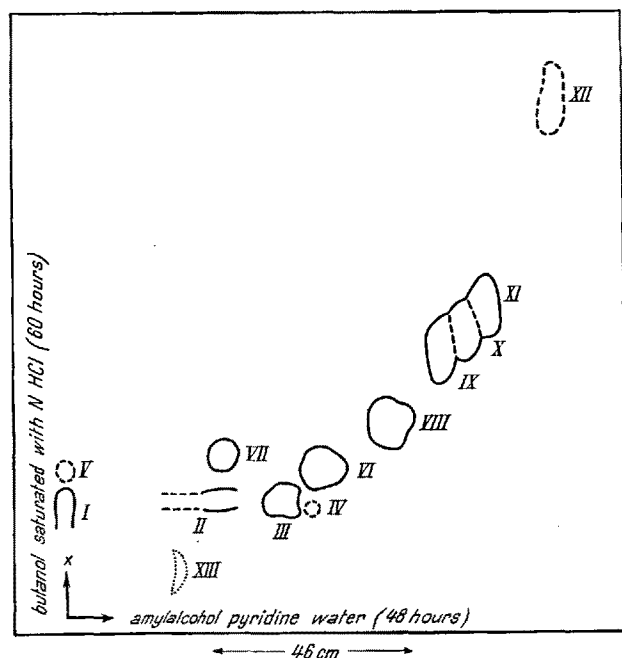


Fig. 2. – At the mark x, 0.01 cm³ of the *Vulgaris*-II extract, corresponding to 0.2 g fresh salivary tissue.

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

The positiveness 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 question¹.

(b) After ultraviolet irradiation, spot VII shows an intense adrenaline-like action on the blood pressure and on various isolated organs. It surely contains octopamine².

¹ V. ERSAPMER, Naunyn-Schmiedeberg's Arch. 200, 60 (1942); Acta pharmacol. 4, 213 (1948). – V. ERSAPMER and G. BORETTI, Exper. 6, 428 (1950).

² V. ERSAPMER, Acta pharmacol. 4, 224 (1948).

(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 generalization of data obtained in one or even in more species of *Octopoda* is therefore quite arbitrary.

V. ERSAPMER 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.

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 experiments¹ leukocyte suspensions were examined as isolated by the SZILARD-method². 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).