higher than that indicated in the Table as ordinarily totally inhibiting the enzyme in epithelioma.

The results demonstrate that in epithelioma, the enzyme under consideration is altered in that it has a higher activity, with different Zn^{++} and O-phenantroline inhibition constants as compared with normal skin enzyme.

Investigations carried out along these lines could be of use for the differential diagnosis in skin tumours.

Glutamate dehydrogenase activity in normal skin and skin epithelioma

		Activity in μmol/g/min	
		Normal	Epithelioma
Control		1.90 ± 0.10	4.45 ± 0.16
+ $ZnSO_4$	$1.4 \times 10^{-4} M$	1.85 ± 0.06	3.29 ± 0.21
	$1.9 \times 10^{-4} M$	0	2.45 ± 0.08
	$2.5 \times 10^{-4} M$	0	0
+ O-phenantroline	$1 \times 10^{-4} M$	1.90 ± 0.07	4.46 ± 0.14
	$5 \times 10^{-3} M$	0.43 ± 0.03	3.55 ± 0.18
	$1 \times 10^{-2} M$	0	0.92 ± 0.12

41 control tests, 41 Zn^{++} tests, 10 O-phenantroline tests.

Résumé. Les auteurs ont étudié l'activité de la glutamate déhydrogénase dans la peau normale et dans les épithéliomes de la peau chez l'homme. Ils ont observé que l'enzyme est dans les tumeurs beaucoup plus active que dans la peau normale. De même, dans l'épithéliome, l'inhibition par le Zn^{++} et la O-phénantroline necessite de plus hautes concentrations que pour la peau normale. Les modifications constatées pourraient servir au diagnostic différentiel des tumeurs de la peau chez l'homme.

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Isozymes of the Pyrophosphatase from the Brain

The existence of isozymes raises interesting problems concerning the possibilities to control the metabolic activity as well as the structure of chromosomic genes. Therefore many researchers have succeeded lately in making evident the 'isozymic spectra' of enzymes involved in the fundamental processes of the vegetal and animal organisms.

In the present paper we report the results obtained on the isozymic structure of the pyrophosphatase (E.C. 3.6.1.1.) in the brain. This enzyme plays a prominent part in the processes of pyrophosphorylation, controlling the concentration of pyrophosphate which appears in numerous metabolic reactions.

So far, data on the existence of the multiple molecular forms of the pyrophosphatase (PPase) are not yet known. G. A. MELONI, A. PESCE and G. C. SCHITO[1] mention that they succeeded in separating 2 forms of PPase from *Pseudomonas aeruginosa*. But as these 2 components could be obtained only by chromatography on DEAE-cellulose and not on Sephadex, the authors infer that they have the same molecular weight, differing only by their electric charge.

Our attempts, regarding the chromatography on Sephadex G-200 of extracts from some animal organs, succeeded in separating many fractions which revealed PPase activity. The enzymogrammes obtained proved the presence of PPase isozymes in tissues.

We show below the results obtained in the case of brain. For that purpose we collected the brain from 8 different species (hen, goose, rabbit, guinea-pig, dog, horse, pig and cow) immediately after slaughter and bleeding and we homogenized it in the ratio 1:10 with a cold saline *Tris* buffer pH 7.2 (*Tris*, HCl $0.05 M$ + NaCl $0.1 M$).

We centrifugated at 1500 *g* for 30 min and after removing the cellular debris we did it again at 9000 *g* for 1 h. The supernatant was filtered through a 2 × 25 cm column filled with Sephadex G-200 equilibrated with the previous

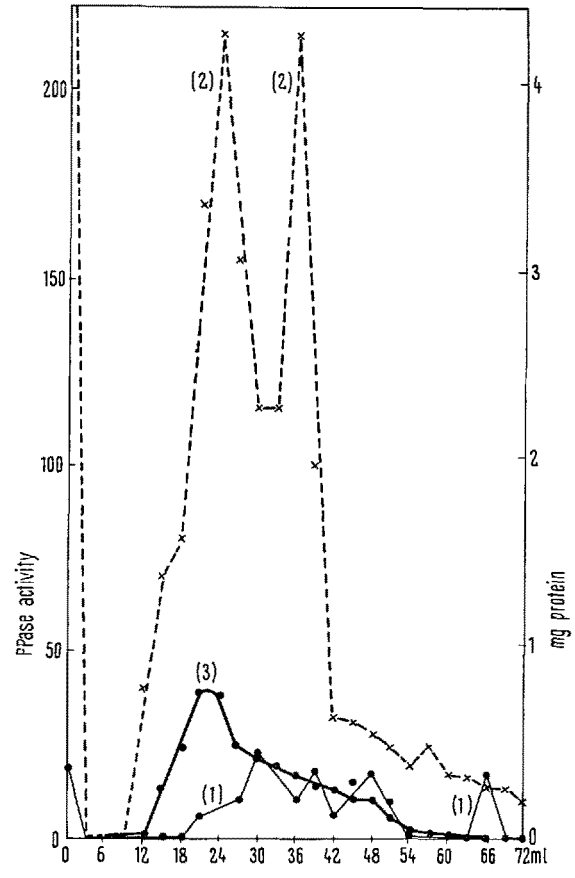

Fig. 1. PPase isozymes of the hen brain. (1) Enzymogram of the brain extract. (2) Proteinogram of the brain extract. (3) Proteinogram of the bovine serum albumin.

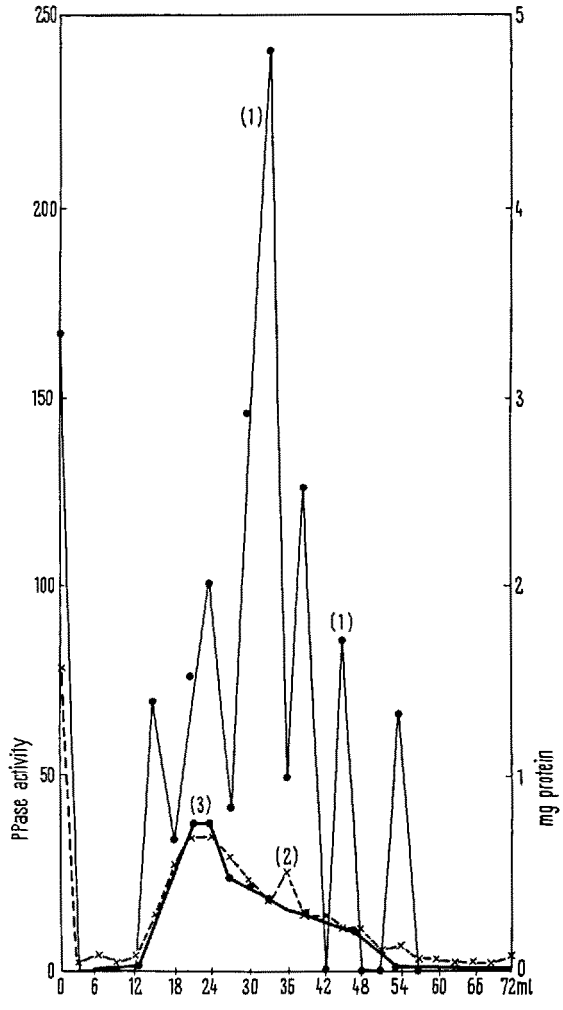

Fig. 2. PPase isozymes of the rabbit brain. (1) Enzymogram of the brain extract. (2) Proteinogram of the brain extract. (3) Proteinogram of the bovine serum albumin.

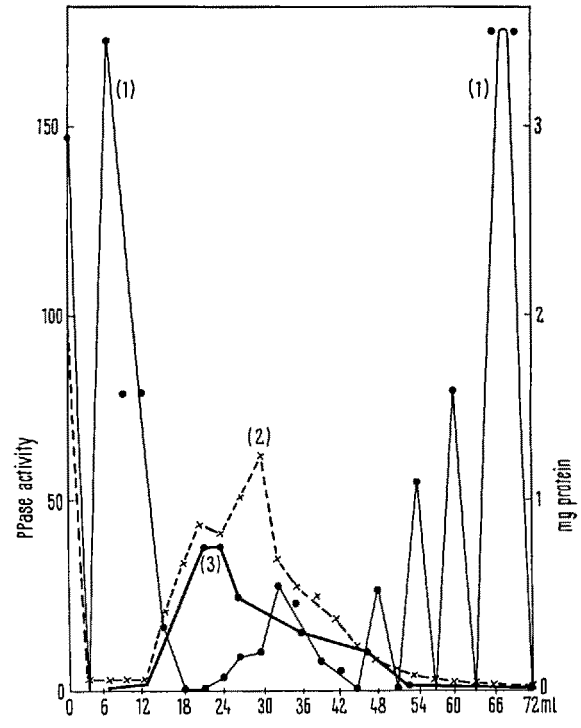

Fig. 3. PPase isozymes of the cow brain. (1) Enzymogram of the brain extract. (2) Proteinogram of the brain extract. (3) Proteinogram of the bovine serum albumin.

buffer and fractions of 3 ml were collected by elution with the same buffer solution.

The PPase activity was determined in every fraction by measuring the quantity of phosphate released from the substratum solution of pyrophosphate 0.0125 M in the presence of magnesium chloride of the same molarity, the value of the optimum ratio [P-P_i/$MgCl_2$] being, for the brain, approximately equal to the unit.

The concentration in protein was determined by means of Folin's reagent with MILLER's technique[2]. The activity was represented in mg phosphorus/g protein.

The activity and protein concentration were plotted against the volume of elution. In the Figures we give the results obtained for only 3 species from the total of 8 worked.

These results show that several protein fractions presenting PPase activity can be obtained through gel-filtration.

Their numbers vary with the species ranging from 3 in the case of guinea-pig to 7 for the dog. The lowest PPase activity was found for the hen's brain. The molecular weight of the fractions and their PPase activity vary with the species. In order to appreciate the molecular weight of the different fractions, the column was calibrated with bovine serum albumin crystallized twice (N.B.C. product molecular weight 66,700).

From data obtained up to now it cannot be specified if the existence of such a big number of isozymes is due to a genetic determinism (polyallelism), or only to histological diversification corresponding to particular activities in different cerebral sectors.

The reproduction of the results obtained for the same species is very satisfactory.

Experiments under way try to establish the kinetic differences of the isozymes separated, as well as the influence of some factors like cross-breeding, metabolic disorders etc. on specific enzymogrammes.

Résumé. On a reussi à séparer par chromatographie sur colonne à Sephadex G-200 plusieurs isozymes de la pyrophosphatase (E.C. 3.6.1.1.) du cerveau chez divers animaux. Les enzymogrammes obtenues sont spécifiques pour chaque espèce.

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25 January 1968.

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[2] G. L. MILLER, Analyt. Chem. *31*, 964 (1959).