increase with the ethyl group, but the marked increase occurs on passing to the propyl derivative, followed by a decline to the butyl derivative. 5-Chloro-1-propyl-HBB (I; R = Pr, R′ = Cl) has high activity and selectivity (Table I), but is less active and usually less selective than PHBB (I; R = Pr, R′ = H)[4]. Each 1-alkyl compound is more active than its corresponding 5-chloro derivative. The latter compounds also have a disadvantage in being less soluble in tissue culture medium. However, prolonged warming will produce satisfactory dispersion even with the butyl derivative up to approximately 100 $\mu$moles/l. Hydrochlorides of the 5-chloro compounds were used in our experiments, but the proportion of dissolved free base depends, of course, on the pH of the medium.

DL- and D-5-chloro-2-(α-hydroxybenzyl)benzimidazoles (I; R = H, R′ = Cl) and the DL-1-alkyl-5-chloro derivatives (I; R = alkyl, R′ = Cl) were obtained by heating under reflux, the appropriate 4-chloro-*o*-phenylenediamine (1 mole) with DL- or D-mandelic acid (1 mole) in 2*M* hydrochloric acid (2.5 moles) for 6 h (for the butyl derivative, 4*M* hydrochloric acid and 12 h were necessary). Those hydrochlorides that separated were removed, and the bases were obtained on neutralizing either the reaction mixtures or aqueous solutions of the hydrochlorides with 3*M* potassium carbonate. Repeated crystallization from aqueous methanol with charcoal treatment gave the benzimidazoles (Table II). D-5-chloro-2-(α-hydroxybenzyl)benzimidazole hydrochloride crystallized from ethanol-ether mixture and had $[\alpha]_D^{17}$ of $-78.6°$ (*c* 0.81 in ethanol), but its base (m.p. 85–100°) was not obtained in a pure state. Our yield of the parent DL-isomer was better than that previously obtained by fusing the diamine with mandelic acid[6]. N-Alkyl-4-chloro-2-nitroanilines were prepared from the alkylamine and 2,5-dichloro-1-nitrobenzene[7]. 4-Chloro-2-nitro-N-propylamine was obtained, in 84% yield, as orange prisms from ethanol, m.p. 49–50° (Anal.-Found: C, 50.4; H, 5.12; N, 13.1; Cl, 16.5. $C_9H_{11}ClN_2O_2$ requires C, 50.4; H, 5.13; N, 13.1; Cl, 16.6%). These nitroanilines were hydrogenated to give the diamines[8]. Hydrogen uptake continued until an excess of up to 21% had reacted. Partial dechlorination may explain the low yields of pure benzimidazoles (Table II).

As both relative selectivities (for poliovirus in *ERK* cells) and solubilities in water of the 5-chloro derivatives are generally inferior to those of the corresponding non-halogenated compounds the 5-chloro derivatives are, at present, potentially of less value in relation to polioviruses than the non-halogenated compounds, in spite of their quite high activities. We are testing other halogen derivatives of HBB, of its 1-alkyl derivatives, and of its optical isomers in order to elucidate the overall structure-activity pattern. Results will be reported elsewhere[8].

*Zusammenfassung.* Die D-isomere Verbindung ist verantwortlich für die Poliovirus-hemmende Aktivität von DL-5-Chlor-2-(α-oxy-benzyl)-benzimidazol. DL-1-Propyl-5-chlor- und DL-1-Butyl-5-chlor-Derivate sind ausserordentlich wirksam zur Verhinderung der Vermehrung der Typen 1, 2 und 3 des Poliovirus; andere 1-Alkyl-5-chlor-Derivate sind ebenfalls aktiv. Die 1-Alkyl-Grundverbindungen sind jedoch wirksamer als deren gleichartige 1-Alkyl-5-chlor-Derivate.

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[5] We have not yet tested these compounds with arboviruses.
[6] W. R. Siegart and A. R. Day, J. Am. chem. Soc. *79*, 4392 (1957).
[7] M. J. J. Blanksma, Recl. Trav. chim. Pays-Bas Belg. *21*, 273 (1902).
[8] The research is supported by the National Fund for Research into Poliomyelitis and other Crippling Diseases.

## Glutamate Dehydrogenase Activity in Normal and Tumoural Skin in Humans

In previous studies, the authors demonstrated that a coenzyme-independent lactate dehydrogenase is active in Ehrlich ascites tumour cells[1,2]. They also found an increased activity of glutamate dehydrogenase (GLDH) in the liver of Guérin-epithelioma rats[3].

In the present work, some of the results concerning GLDH activity in normal skin and skin epithelioma will be discussed.

*Materials and methods.* Samples of skin and tumoural tissue (spindlecell epitheliomas and basaliomas) were homogenized in 0.9% NaCl with $7 \times 10^{-4}M$ EDTA. The supernatant resulting from a 10-min centrifugation (1800 *g*) at low temperature, was used for testing GLDH activity in 340 nm[4] with a VSU 1 K Zeiss Jena spectrophotometer.

*Results and discussion.* As seen in the Table, GLDH activity is higher in skin epithelioma than in normal skin in man. The enzyme is inhibited by $Zn^{++}$ ions and O-phenantroline both in normal skin and in skin epithelioma. While in normal skin there is a total $Zn^{++}$ inhibition with $1.9 \times 10^{-4}M$ $ZnSO_4$, in skin epithelioma the activity is found to be more than 50% of the original enzyme activity in tumours, and also exceeds that of normal skin. Total inhibition was only noted in epithelioma at a concentration of $2.5 \times 10^{-4}M$ $ZnSO_4$. While in normal skin $1 \times 10^{-2}M$ O-phenantroline totally inactivates the enzyme, in epithelioma with the same concentration of O-phenantroline, one fifth of the original activity is still found. It should be mentioned that in one instance of epithelioma, inactivation was not obtained even when $Zn^{++}$ and O-phenantroline concentrations were 100 times

[1] L. Ababei and S. Sarkar, Acta biol. med. germ. *14*, 128 (1965).
[2] S. Rapoport, L. Ababei, C. Wagenknecht and S. Sarkar, Nature *208*, 185 (1965).
[3] M. Trandafirescu and L. Ababei, Acta biol. med. germ. *17*, 250 (1966).
[4] Ellen Schmidt, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer; Verlag Chemie, Academic Press, New York and London 1965), p. 752.

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 | | $1.90 \pm 0.10$ | $4.45 \pm 0.16$ |
| + $ZnSO_4$ | $1.4 \times 10^{-4} M$ | $1.85 \pm 0.06$ | $3.29 \pm 0.21$ |
| | $1.9 \times 10^{-4} M$ | 0 | $2.45 \pm 0.08$ |
| | $2.5 \times 10^{-4} M$ | 0 | 0 |
| + O-phenantroline | $1 \times 10^{-4} M$ | $1.90 \pm 0.07$ | $4.46 \pm 0.14$ |
| | $5 \times 10^{-3} M$ | $0.43 \pm 0.03$ | $3.55 \pm 0.18$ |
| | $1 \times 10^{-2} M$ | 0 | $0.92 \pm 0.12$ |

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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*19 January 1968.*

## 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

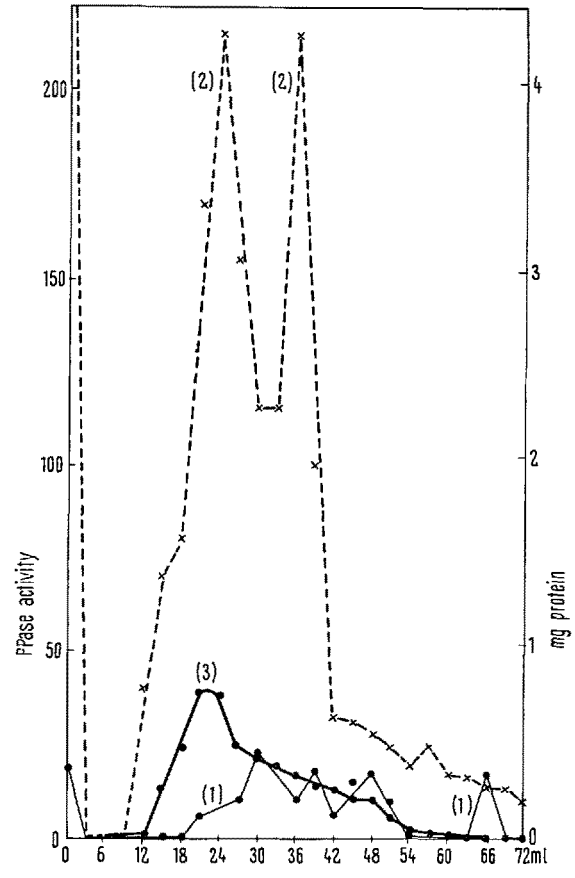


Fig. 1. PPase isozymes of the hen brain. (1) Enzymogram of the brain extract. (2) Proteinogram of the brain extract. (3) Proteinogram of the bovine serum albumin.