causing inhibition of non-choline esterases[6,7]. This indicates that, at a concentration of 10^{-4} M, it is specifically cholinesterase that is inhibited, suggesting that the higher concentration would be needed for the penetration of the inhibitor through the intact cell membrane and for the maintenance of an effective intracellular concentration of the inhibitor broken down by cholinesterases[8].

The 2 inhibitors used in the present study caused identical effects in the differentiating sympathicoblasts, thus further emphasizing the role of acetylcholinesterase inhibition as the basic cause for the changes.

The mesenchymal elements in the cultures presented an interesting opportunity to compare the effects of the inhibitors on sympathicoblasts and non-neural cells in the same culture. The effects proved to be neuron-specific. Moreover, is seemed not to be a question of an acute toxic influence of the inhibitors on nerve cells, but of an inhibition of growth and differentiation of sympathicoblasts appearing in the course of development in culture. The results of the present study strongly suggest that cholinesterases play a significant role in the early differentiation of the sympathicoblasts.

6 L. H. Easson and E. Stedman, Biochem. J. *37*, 1723 (1937).
7 B. Mendel, D. B. Mundell and H. Rudney, Biochem. J. *37*, 473 (1943).
8 L. C. Post, Biochim. biophys. Acta *250*, 121 (1971).

Lysosomal enzyme release associated with the invasion of rat liver by Novikoff hepatoma

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Summary. The lysosomes of both Novikoff hepatoma and liver from Novikoff hepatoma-bearing rats were found to be relatively intact structurally, lower in acid phosphatase activity, greatly depleted in number but with nearly normal membrane integrity when compared with normal liver.

Mounting evidence indicates that lysosomal enzyme release may accompany malignant growth and invasion. On the basis of enzyme activity studies performed on mouse interstitial tumor fluid, Sylvén has postulated that leakage of active lysosomal enzymes, from tumor and/or host, cells, may occur[2]. Pure lines of cultured tumor cells release or secrete enzymic proteins[3] and Carević et al. have demonstrated that acid phosphatase release accompanies the course of progressive infiltration of the liver by leukemia[4]. Our objective was to characterize release possibly associated with the solid form of Novikoff hepatoma (an extensively documented, highly invasive tumor[5]), by comparing the levels of the lysosomal marker, acid phosphatase, in control liver with the levels in tumor and tumor-invaded rat liver. These activities were correlated with lysosomal permeability, to provide a measure of membrane leakage. Finally, a histochemical approach enabled us to correlated the distribution of lysosomes in tumor, tumor-invaded liver and normal liver with the cellular distribution of acid phosphatase activity.

Materials and methods. Male Sprague-Dawley-Holtzman rats weighing 250–350 g were provided with Purina Lab Chow and water ad libitum. Over a period of 11 weeks, Novikoff hepatoma (kindly provided by Dr Ernest Borek,

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2 B. Sylvén, in: Endogenous Factors Influencing Host-Tumor Balance, p. 271. University of Chicago Press, Chicago 1967.
3 B. Holmberg, Cancer Res. *21*, 1386 (1961).
4 D. Carević, V. Šverko, M. Boranić and V. Prpić, Experientia *30*, 241 (1974).
5 A. B. Novikoff, Cancer Res. *17*, 1010 (1957).

Free, total and unsedimentable acid phosphatase acrivities in whole liver homogenates of normal and Novikoff hapta-bearing rats and in whole tumor homogenates. Lysosomal membrane permeability to substrate (alpha-napthol acid phosphate) and to acid phosphatase for the same 3 tissues

Tissue homogenate	Acid phosphatase activity (μmoles P/15 min/g wet weight)			Lysosomal membrane permeability	
	Free[a]	Total[b]	Unsedimentable[c]	Enzyme[d] (acid P'ase)	Substrate[e] (γ-napthol P)
Control liver*	1580 ± 241 (44)	2070 ± 292 (44)	60.3 ± 6.9 (29)	2.91 ± 0.53	75.5 ± 16.3
Liver of rat bearing Novikoff hepatoma*	423 ± 38 (48)	552 ± 34 (49)	46.7 ± 12.1 (29)	8.5 ± 2.3	74.6 ± 9.6
Novikoff hepatoma**	60.5 ± 3.2 (51)	90.0 ± 4.5 (51)	9.76 ± 1.2 (26)	10.8 ± 1.4	63.2 ± 5.6

Values for enzyme activity are computed as means ± SEM. Numbers of experiments in parentheses. * 1 test animal utilized in each experiment. ** Duplicate experiments performed on each test animal. [a]) Free activity – enzyme activity in 0.25 M sucrose homogenate; [b]) total activity – enzyme activity following homogenization in 0.25 M sucrose containing 1% Trition X-100 detergent, to cause complete release of enzyme activity from the lysosomes; [c]) unsedimentable activity – the 0.25 M sucrose homogenate was centrifuged at 270,000 × g for 1 h, and the enzyme activity was measured in the supernatant fraction. [d]) The ratio of unsedimentable to total activity (expressed in percent) gives the lysosomal membrane permeability to acid phosphatase. [e]) The ratio of free minus unsedimentable activity to total minus unsedimentable activity (expressed in percent) gives the lysosomal membrane permeability to γ-napthol acid phosphate.

University of Colorado Medical Center, Denver) was serially transplaned into a total of 50 animals. Studies were conducted on 2–8-day-old transplants. Immediately following decapitation, randomly selected samples of both liver and tumor in addition to kidney which served as a histochemical control were fixed in formal-calcium[6], sectioned on a cryostat and stained for lysosomes using the method of Barka and Anderson[7]. The remaining liver and tumor tissue was rapidly cooled in ice-cold 0.25 M sucrose, minced with scissors and separately homogenized, using a Potter-Elvehjem glass-teflon homogenizer. The homogenates were assayed for acid phosphatase, the most widely used marker enzyme for lysosomes[8], using the method described by Allen and Gockerman[9], with alphanapthyl acid phosphate as substrate. The results are reported as free, total and unsedimentable activities (μmoles of inorganic phosphate liberated/15 min gm wet weight). The ratio of unsedimentable to total activity was used as an index for in vivo lysosomal membrane permeability to acids phosphatase, i.e. as a measure of leakage of enzyme activity[4], although we appreciate the possibility that unsedimentableactivity could be due to rupture of lysosomes during homogenization or to enzyme present in the cytoplasmic sap from the time of its synthesis. The free particulate activity was calculated by substracting the unsedimentable activity from the free activity and the total particulate activity was calculated by substracting the unsedimentable activity from the total activity. The ratio of free particulate to total particulate activity was then used as an index for in vivo lysosomal membrane permeability to substrate[10].

Results. In both Novikoff hepatoma and in the liver of tumor-bearing rats the free, total, and unsedimentable acid phosphatase activities were lower than in control liver (table). The decrease in free and total activities was especially pronounced in the hepatoma (25fold less than controls) but was also significantly lower in the invaded

6 A. Pearse, in: Histochemistry, p. 787. Little, Brown and Co., Boston 1961.
7 T. Barka and P. J. Anderson, J. Histochem. *10*, 741 (1962).
8 A. B. Novikoff and E. Holtzman, in: Cells and Organelles, p. 121. Holt, Rinehart and Winston, Inc., New York 1970.
9 A. M. Allen and J. Gockerman, Ann. N. Y. Acad. Sci. *121*, 616 (1964).
10 C. de Duve and H. Beaufay, Biochem. J. *73*, 610 (1959).

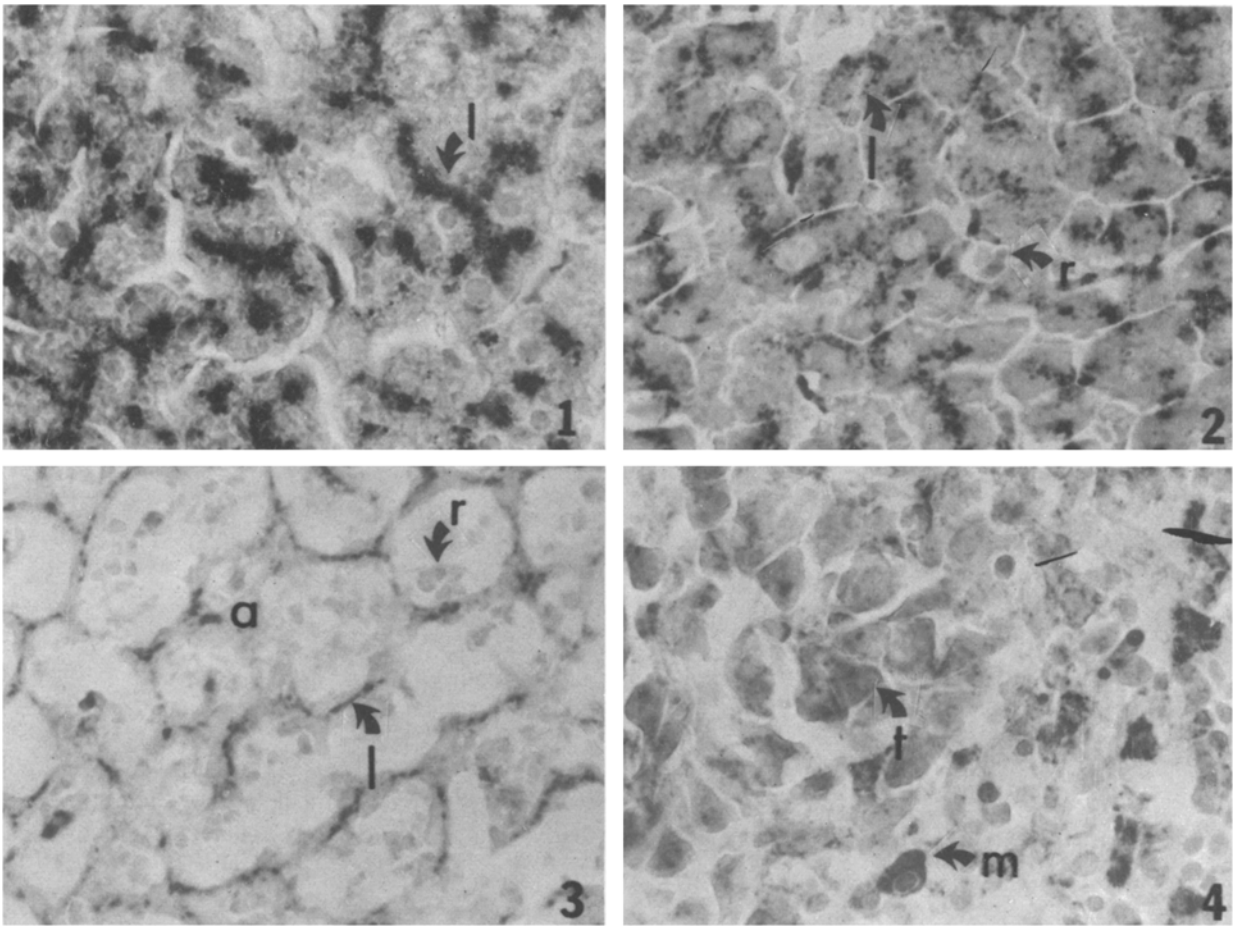

Fig. 1. Control liver. Large numbers of lysosomes appear as dense deposits of reaction product in the peribiliary regions of the liver cells (l). ×640.
Fig. 2. Liver of a Novikoff heptoma-bearing rat. Liver is not invaded by tumor. Lysosomal concentration is less than in controls, as evidenced by the ability to visualize individual lysosomes (l). Incipient hemorrhage is manifest in the presence of an increased number of interstitial red blood cells (r). ×640.
Fig. 3. Liver of a Novikoff heptoma-bearing rat. Liver is being actively invaded at its periphery by metastasizing tumor (not shown). Cellular atrophy is extremely pronounced (a) and interstitial red blood cells are profuse (r). Lysosomal activity is fairly low (l). ×640.
Fig. 4. Novikoff heptoma. The paucity of acid phosphatase activity in tumor cells (t) stands in marked contrast to the intense staining found in nearby proliferating macrophages (m). ×640.

liver (4fold less than controls). The lysosomal membrane permeability to acid phosphatase was 4 times higher than controls in the tumor and 3 times higher in tumor-invaded liver however, approximately $^{9}/_{10}$ of the total tissue acid phosphatase activity remained membrane-sequestered. Lysosomal membrane permeability to substrate in tumor and tumor-invaded liver was not significantly different from control liver, suggesting that loss of membrane integrity in intact lysosomes, is not associated with either the cancerous state or with host response to invasive activity.

Histochemically, fewer lysosomes were observed in Novikoff hepatoma cells (figure 4) than in normal liver cells (figure 1). In many tumor sections the only acid phosphatase activity to be detected was that associated with the white blood cells. In the tumor-invaded liver (figure 3) the lysosomes were more plentiful than in the tumor, but less abundant than in normal liver, and cellular atrophy and evidence of hemmorhage was pronounced. An intermediate condition was seen in the livers of tumor bearing rats that had not been actively invaded by hepatoma (figure 2).

Discussion. Our data allows us to derive several conclusions concerning the nature of lysosomal enzyme release, if such a phenomenon indeed occurs, in Novikoff hepatoma and tumor-invaded liver. The low intracellular levels of acid phosphatase present in these tissues indicates that, if any loss of lysosomal enzyme to the extracellular space occurs, it is not totally compensated for within the cell, e.g. by increased protein synthesis. Since the lysosomes of these tissues show nearly normal membrane integrity, leakage from intact but structurally damaged lysosomes (a common mechanism in various pathological processes other than cancer[10–12]) cannot be evoked as an explanation for primary enzyme loss. An hypothesis, based on our observation of decreased numbers of morphologically normal lysosomes in both the cancerous tissues analysed, would support a mechanism of selective lysosomal rupture. Indeed, such a mechanism might explain the extensive necrosis that we observed in the host liver tissue, since selective lysosomal rupture is known to cause the destruction of cellular and extracellular materials[8].

11 H. Beaufay and C. de Duve, Biochem. J. *73*, 604 (1959).
12 J. L. E. Ericsson, V. T. Brunk and B. Arborgh, Histochem. J. *6*, 63 (1974).

Direct intercellular contacts at the ectomesodermal interface during the duck embryologic preen gland development[1]

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Summary. The epidermis, which lines the uropygial invaginations and which then forms the primary and secondary buds, is separated from the mesenchyma by an uninterrupted basal lamina. Between the end of the internal morphogenesis and the beginning of the secretory activity, direct intertissular contacts are established through the gaps in the basal lamina. They appear to be related to the induction of glandular differentiation.

Numerous studies have shown that the morphogenetic development of different organs depends on the interaction between epithelial and mesenchymal components of organ rudiments. The available data concerning this interaction have been the subject of several reviews[2,3], but the transmission mechanism of the inductive signals have not been clearly defined. The suggestion that diffusion of high-molecular-weight material might mediate the inductive effects without direct contacts between the mesenchymal and epithelial cells was originally based on transfilter experiments believed to exclude cell contacts[4]. However, a positive correlation was observed between successful transfilter mouse kidney tubules induction and the establishment of a direct apposition of cytoplasmic processes from the interacting cells in the filter channels[5,6]. Direct cell contacts occurring during epithelio-mesenchymal interaction in the normal kidney tubules induction in vivo support the hypothesis that direct intercellular contact is the induction mechanism in this system[7]. Such contacts are observed in several developing tissues: foetal mouse liver[8], tooth buds in the cat[9], in the rat[10], human regenerating skin[11], rat duodenal mucosa[12] and rat submandibular gland[13].

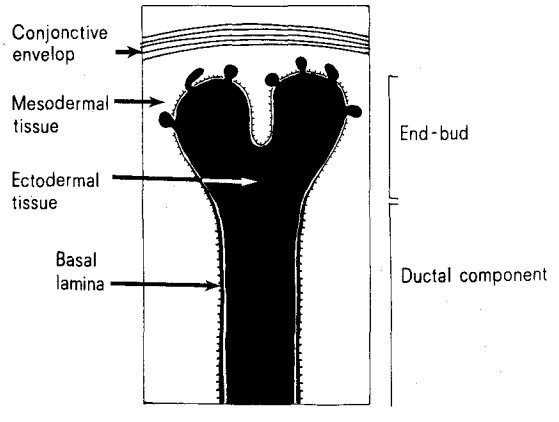

Fig. 1. Schematic representation of the uropygial tubule terminal part at the end of the morphogenesis, showing the zone of direct ecto-mesodermal contacts.

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2 C. Grobstein, Nat. Cancer Inst. Monogr. *26*, 279 (1967).
3 K. Kratochwill, in: Tissue Interactions in Carcinogenesis, p. 1–47. Ed. D. Tarin. Academic Press, New York 1972.
4 C. Grobstein, Exp. Cell Res. *10*, 424 (1956).
5 J. Wartiovaara, S. Nordling, E. Lethonen and L. Saxen, J. Embryol. exp. Morph. *31*, 667 (1974).
6 E. Lethonen, J. Wartiovaara, S. Nordling and L. Saxen, J. Embryol. exp. Morph. *33*, 187 (1975).
7 E. Lethonen, J. Embryol. exp. Morph. *34*, 695 (1975).
8 R. A. Rifkind, D. Chui and H. Epler, J. Cell Biol. *40*, 343 (1969).
9 E. Pannese, J. Ultrastruct. Res. *6*, 186 (1962).
10 E. Kallenbach, J. Ultrastruct. Res. *35*, 508 (1971).
11 R. Ross and G. Odland, J. Cell Biol. *39*, 152 (1968).
12 M. Mathan, J. A. Hermos and J. S. Trier, J. Cell Biol. *52*, 577 (1972).
13 L. S. Cutler and A. P. Chaudhry, Dev. Biol. *33*, 229 (1973).