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Histochemical Distribution of Alkaline Phosphatase in Dog Liver After Experimental Biliary Obstruction.

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Gomori[1] was the first to notice that the bile capillaries in the liver of some species showed marked phosphatase activity. The constant increase of serum alkaline phosphatase after ligation of the bile ducts in the dog[2] and the fairly regular phosphatase activity of the bile capillaries in the dog liver suggested that a study be made of the behavior of alkaline phosphatase activity in the liver after ligation of the bile ducts.

Material and Methods. In 9 mongrel dogs the common bile duct was ligated under nembutal anesthesia after an initial biopsy had been taken. In 2 acute experiments the cystic duct also was ligated. Serum phosphatase activity was estimated with Bodansky's method[3] using a Leitz photocolorimeter. Gomori's method[4] as modified by Kabat and Furth[5] was used for the histochemical demonstration of alkaline phosphatase activity. Alternate sections were routinely incubated for 2 and 14 hours. When indicated, the incubation period was shortened to 30 and 60 minutes. No counterstain was employed.

Results. The changes in the dog liver after duct ligation as seen in slides stained with hematoxylin-eosin, were essentially similar to those previously described.[6,7] They consisted of hyperemia, atrophy of liver cells in the central fields, occurrence of bile thrombi, predominantly in the central fields and very occasional occurrence of small foci of necrosis. Bile pigment was seen in the liver cells mainly around central fields. Kupffer cells frequently contained bile pigment.

Normal Liver. (2-hour incubation). With the exception of bile capillaries and larger bile ducts, there was only very faint phosphatase activity in all other structures. The cytoplasm of the liver cells took on a faint grayish color. Most of the nuclei could be recognized due to some staining of the nuclear membrane and of the nucleoli. Only occasionally was there activity in the endothelium of the sinusoids near the periportal areas. Larger bile ducts in the periportal areas showed marked phosphatase activity. The bile capillaries were outlined as fine, black, lines sometimes showing a definite lumen (Fig. 1). They showed frequently, the well-known tortuous appearance. In some areas, they were seen between 2 liver cell cords (trabecular), in others they formed networks apparently surrounding single liver cells. From the trabecular capillaries intercellular branches went off at various angles. On cross section, they appeared as small round black dots or circles. Very occasionally, the transition of a capillary into a bile duct could be seen. The activity of the bile capillaries was somewhat more distinct near the periportal fields than around central areas. There was, however, some variation in different livers.

Normal Liver. (14-hour incubation). The staining of the cytoplasm and of the nuclei became more intense. The activity of the sinusoidal lining as well as of their nuclei became marked. Connective tissue cells and occasional mononuclear cells in the periportal fields showed also some activity. The intensification of the reaction was moderate in

1 Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, **17**, 71.

2 Armstrong, A. R., King, E. F., and Harris, R. I., *Canad. M. A. J.*, 1934, **31**, 14.

3 Bodansky, A., *Am. J. Clin. Path.*, Techn. Suppl., 1937, **1**, 51.

4 Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.

5 Kabat, E. A., and Furth, J., *Am. J. Path.*, 1941, **17**, 303.

6 Ogata, T., *Beitr. Z. Path. Anat. u. Z. Allgem. Pathol.*, 1913, **55**, 236.

7 Hiyeda, D., *Beitr. Z. Path. Anat. u. Z. Allgem. Pathol.*, 1925, **73**, 541.

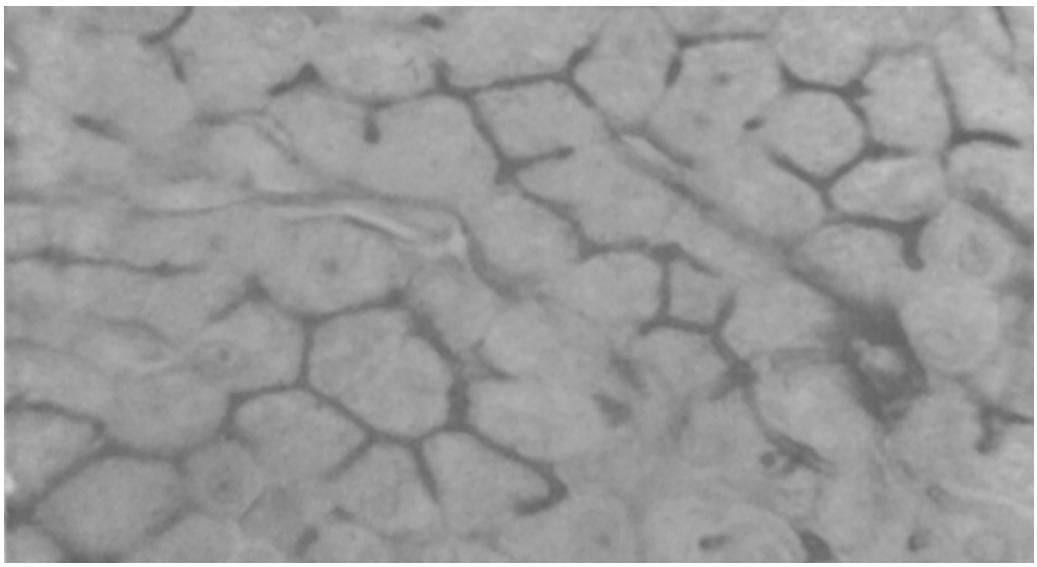

FIG. 1.
Alkaline phosphatase activity in the normal dog liver. The bile capillaries show prominent staining. Incubation in the substrate mixture for 2 hrs. × 800.

some, quite marked in other livers. In the latter group, the bile capillaries also showed stronger staining and occasionally their outline became hazy. The concomitant staining of the other structures made the activity of the bile capillaries less conspicuous.

Effect of Duct Ligation. (3-4 days). Two dogs died after 3-4 days because of extensive wound infection. One was killed 4 days after ligation. This dog showed a rise of blood serum phosphatase from 2.4 to 43.2 units. In sections incubated for 2 hours a considerable number of bile capillaries were wider than normal. Some showed a hazy outline. There was some increase in cytoplasmic phosphatase. After longer incubation, this increase became more striking and the cytoplasm showed many fine, black granules. The 2 dogs that died showed very marked dilatation of the bile capillaries which were considerably widened and not clearly demarcated, from the surrounding cytoplasm. In addition, considerable increase in cytoplasmic activity was seen.

One Week. One dog was killed after 8 days. Serum phosphatase rose from 1.1 to 47.6 units. In sections incubated for 2 hours, there was very marked dilatation of the bile capillaries which stood out much clearer than in the biopsy. Many of these showed a distinct lumen. The demarcation from the cytoplasm was not sharp in a large number of these capillaries. The cytoplasm showed increase in activity somewhat more pronounced around the periportal fields. The bile ducts in the periportal fields showed marked increase in activity and their lumen was filled by intensely dark staining material. After longer incubation, the reaction became so intense that the structures could hardly be differentiated.

Two Weeks. Two dogs were killed after 2 weeks. In one the serum phosphatase rose from 1 to 36 units. In this dog, dilatation of the bile capillaries occurred predominantly at the periportal fields. The second dog showed very widespread dilatation of the bile capillaries. While some of them still showed their clear outline, many became very irregularly demarcated and hazy. The increase in cytoplasmic activity was very conspicuous.

Three Weeks. One dog was killed 3 weeks after ligation. His initial serum phosphatase of 1.4 units rose to 27.6 units in 8 days, and to 32.2 units in 14 days. The increase in phosphatase activity was so intense that sections incubated longer than one hour could

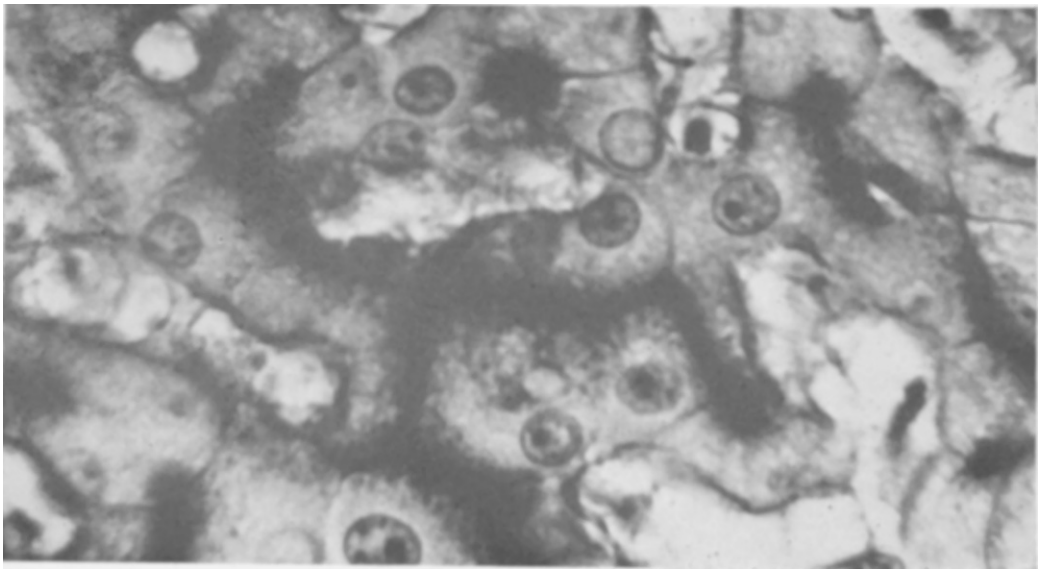

FIG. 2.
Alkaline phosphatase activity in the liver of a dog, 3 weeks after ligation of the common bile duct. Conspicuous widening of many bile capillaries. Incubation in the substrate mixture for 30 minutes. ×800.

not be used. Widening of the bile capillaries was extremely marked in many areas (Fig. 2). It appeared as if large amounts of phosphatase were accumulated in liver cells near the bile capillaries. In this way, the width of the bile capillaries, on cross section, appeared to be 4 to 5 times larger than normal. Occasionally, a lumen could be made out. In contrast to the conspicuous changes in many of the capillaries, some were of fairly normal appearance.

Acute Experiments. In 2 dogs after initial biopsy, the cystic and common ducts were ligated and biopsies were taken after 30, 60, 90, and 120 minutes. No changes were seen in serum phosphatase in one of the dogs in which this examination was carried out. After one hour the bile capillaries stood out somewhat more distinctly and showed occasional light dilatation. There was also a very slight increase in cytoplasmic activity.

Comment. The source of the increased amount of alkaline serum phosphatase which occurs in obstructive and to a lesser degree in parenchymatous jaundice is still controversial.

The accumulation of phosphatase around bile capillaries is in marked contrast to the diffuse increase of enzymatic activity which occurs in the liver of protein depleted mice and rats and to a lesser degree in starvation.[8] It can best be interpreted as retention of alkaline phosphatase in liver cells caused by external obstruction. No significant increase in alkaline phosphatase activity is seen, on the other hand, in necrotic liver cells.[8] The behavior of the histochemically demonstrable phosphatase activity under various experimental conditions therefore, supports the opinion of those who believe[9,10] that the increase of serum phosphatase in liver damage is due to disturbed excretion but not to increased production of alkaline phosphatase in the liver cells.[11]

Summary. Bile capillaries show distinct phosphatase activity in the normal dog liver. This property can be used for their microscopic demonstration. After ligation of the common bile duct there is not only marked

8 Wachstein, M., *Arch. Path.*, 1945, **40**, 57.

9 Armstrong, A. R., and Banting, F. G., *Canad. M. A. J.*, 1935, **33**, 243.

10 Maddock, S., Schmidt, G., and Thannhouser, S. F., *Federation Proc.*, 1942, **1**, 181.

11 Bodansky, A., *Enzymologia*, 1937, **3**, 258; PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 800.

dilatation of the bile capillaries but also apposition of phosphatase around them which increases with the duration of the experiment. The changes in the distribution of alkaline phosphatase in histochemical preparations favor the assumption that the increase of serum phosphatase in liver damage is due to the inability of the liver cells to excrete the enzyme rather than to increased production in the liver itself.

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Morphologic Effects of DDT on Nerve Endings, Neurosomes, and Fiber Types in Voluntary Muscles.*

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The effect of DDT (2, 2 bis(p-chlorophenyl)-1,1,1-trichloroethane) on the voluntary neuromuscular apparatus in the living, intact animal is unknown. Lewis and Richards[1] state that DDT does not affect the growth of various cells in 7- to 8-day chick embryos and in a 1-day rat in hanging drop cultures. They are unable to explain the apparent paradox of the toxicity of DDT to intact, living animals and its non-toxicity on their isolated cells in hanging drop cultures. Yeager and Munson[2] have presented physiological evidence for a possible site of action of DDT in bringing about repetitive discharges of nerve impulses into the muscles of the roach, *Periplaneta americana,* which indicates that the site (or sites) referred to is that region of a nerve which lies between the origin of its fibers in the ventral nerve cord and the termination of its fibers in the leg, exclusive of the origin and the endings, that is, the myoneural junctions. Their results suggest that DDT can act more readily on motor than on sensory nerves and, further, that DDT stimulates motor nerves somewhere along their course between the cells of origin in the ventral nerve cord and the nerve endings in muscle.

The peripheral effects of DDT on the living, nerve-intact muscle, nevertheless, are visibly evident in chameleons and rats, in the persistent, involuntary, clonic contractions and occasional epileptoid convulsions and violent tremors of the entire voluntary musculature until complete flaccid or spastic paralysis and death occur. The purpose of this paper, therefore, is to demonstrate the striking morphologic changes observed in the voluntary neuromuscular apparatus of chameleons and rats during the transmission of a quantitative increase of neurogenic substances into the myoplasm of some muscle fibers affected by DDT.

Methods. A quick-killing emulsion[1] was prepared which contained 1% DDT, 8% olive oil, 1% gum arabic, and 90% Locke's solution; 2 cc of this emulsion was injected intraperitoneally into 48 white rats *(Mus norvegicus),* average weight 250 g, and 0.1 cc into 100 summer pseudo-chameleons *(Anolis caroliniensis),* average weight 3 to 8 g. Eight rats were selected at 12-hour intervals after the injection of DDT for microscopic study of the gastrocnemius muscle, sciatic nerve, and spinal cord. Various histologic technics were employed for the study of Nissl substance, myelin sheath, axis cylinders, motor end plates, and liposomes in muscle. Ten chameleons were selected also, but at 2-hour

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2 Yeager, J. F., and Munson, S. C., *Science,* 1945, **102**, 305.