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Parathyroid Hormone and Liver Regeneration[1] (38221)

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Previous studies have shown that parathyroid hormone is a regulator of liver regeneration (4, 9, 13). Removal of the parathyroid glands reduces the initiation of hepatocyte deoxyribonucleic acid (DNA) synthesis and proliferation following partial hepatectomy (4, 9, 13), an effect which can be overcome by the injection of parathyroid hormone immediately after the liver resection (4). Thus, it was suggested that partial hepatectomy might stimulate the secretion of parathyroid hormone which, in turn, would proliferatively activate the normally noncycling liver parenchymal cells (4, 9). This proposal seemed to be supported strongly by the fact that partial hepatectomy causes an initial, transient (12–16 hrs), hypocalcemia (4, 9, 13), which is the physiological signal for the secretion of parathyroid hormone (2, 10).

In the present communication, we present new evidence which eliminates the possibility of a burst of parathyroid hormone secretion being involved in the proliferative activation of liver cells. On the other hand, we will show that the hormone is needed to continuously maintain the potential of liver parenchymal cells to initiate DNA synthesis and enter mitosis after they have been activated by some as yet unknown process unleashed by partial hepatectomy.

Materials and Methods. The uptake of ^{3}H-thymidine into cell nuclei and mitotic activity were used to evaluate the regenerative response of parenchymal liver tissue to partial hepatectomy in normal, parathyroidectomized (PTX), thyroparathyroidectomized (TPTX) and sham PTX-TPTX animals. All studies were carried out with males (180–220 g) of a specific-pathogen-free strain of Sprague–Dawley rat which were bred in this laboratory. The experimental design and procedures used in this study have been described previously (9).

The experiments began by preparing groups of PTX or TPTX rats. The animals were anaesthetized with Fluothane (Ayerst Laboratories, Montreal) and their thyroid regions surgically exposed. To PTX the animals, only the parathyroid glands were destroyed and this was done by electrocautery. To TPTX the animals, the entire thyroid–parathyroid complex was removed surgically. Sham controls were subjected to the same surgical manipulations, but the glands were not removed. Any PTX or TPTX rat which had a total plasma calcium concentration of more than 7 mg % at sacrifice was regarded as an operational failure and was not used.

At specific intervals after the PTX, TPTX, or sham operations, the median and left anterior lobes of the liver were removed. This partial hepatectomy, which involved 68% of the liver mass, was carried out under aseptic conditions according to Higgins and Anderson (3).

All animals were given food (Laboratory Chow, Ralston Purina of Canada, Ltd., Woodstock, Ontario) and tap water *ad libitum* during the experimental period. This condition differs from previous experiments (4, 9) in which the animals were starved. However, feeding was found not to alter the basic effect of removal of the parathyroid glands on liver regeneration (cf. 4, 9)

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and had the additional benefit of reducing death from hypocalcemic tetany caused either by parathyroidectomy alone or its combination with partial hepatectomy.

After liver resection, the rats were sacrificed at predetermined times for samples of blood and liver tissue. Each animal was anaesthetized with ether and 4–5 ml of blood removed from the abdominal aorta with a syringe and hypodermic needle moistened with heparinized saline. The total calcium concentration in the plasma of these blood samples was determined within two hours of isolation by titration with EGTA (ethylene-*bis*(oxyethylenenitrilo)tetraacetic acid) using a Fiske automatic fluorometric titrator (Fiske Assoc. Inc., Uxbridge, Mass.) with calcein as the indicator.

Immediately after the withdrawal of blood, the right anterior lobe of the liver (1) was removed for autoradiography and histology; this lobe was referred to previously (4, 9, 13) as the right lateral lobe. Blocks of liver tissue were fixed in formol-acetic acid, embedded in paraffin and sectioned at 5 μm. To measure the mitotic activity, sections, strained with hematoxylin and tartrazine, were scored for the proportion of parenchymal cell nuclei which were in prophase, metaphase, anaphase, and telophase (mitotic index, 9).

To determine the proportion of liver parenchymal cells synthesizing DNA, autoradiographs of liver tissue were prepared from animals which had been injected with ^{3}H-thymidine (1.0 μCi/g; sp. act. 20 Ci/mmole; New England Nuclear Corp., Boston) 1 hr before sacrifice. Histological sections of these liver samples were dewaxed, washed 3 times in a 10 m*M* solution of unlabeled thymidine in distilled water, covered with nuclear track emulsion NTB-2 (Eastman Kodak, Rochester, N. Y.) and stored at 4° for 14 days. The autoradiographs were then developed and the underlying cells stained with hematoxylin and eosin. The parenchymal cell nuclei in these preparations were scored to obtain the ^{3}H-thymidine labeling index (9).

Before describing the results of the present series of experiments, it is important to note that in this study the peak period of DNA synthesis occurred at or near 26 hr after partial hepatectomy (Fig. 2) rather than at 22 hr as previously observed (9). This shift occurred suddenly and inexplicably in our animals in the summer of 1972 and has remained consistent since that time. Simultaneously with this shift, the absolute values for DNA synthesis and mitotic ac-

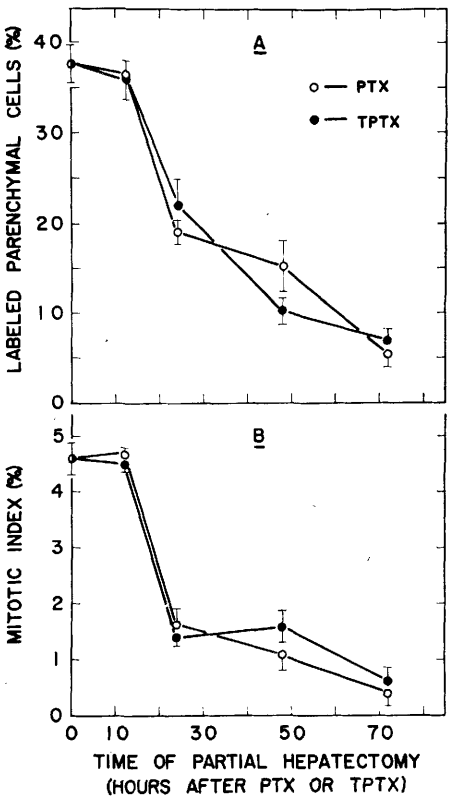

FIG. 1. The ability of partial (68%) hepatectomy to induce DNA-synthetic (A) and mitotic (B) activity in the hepatocytes of the right anterior lobe of the liver of rats which had been parathyroidectomized (PTX) or thyroparathyroidectomized (TPTX) at various times before the liver resection. DNA-synthetic activity is expressed as the percentage of parenchymal liver cells which incorporated ^{3}H-thymidine at 26 hr after the partial hepatectomy. Mitotic activity is expressed as the total percentage of parenchymal cells in prophase, metaphase, anaphase and telophase at 28 hr after the partial hepatectomy. The zero time points represent the values obtained in normal partially hepatectomized, rats. Each value is the mean ± S.E.M. of 6–10 animals.

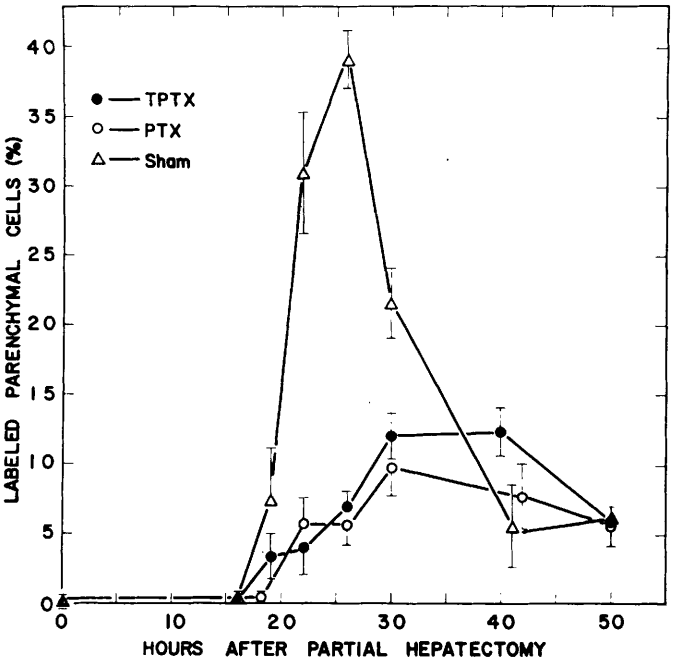

FIG. 2. The percentage of parenchymal cells in the right anterior lobe of the liver which incorporated ^{3}H-thymidine at various times after partial hepatectomy in rats which were parathyroidectomized (PTX), thyroparathyroidectomized (TPTX) or sham-operated 72 hr before the partial hepatectomy. Each value represents the mean ± S.E.M. of 5–7 animals.

tivity following partial hepatectomy in both normal or parathyroidectomized rats also changed (cf. 4, 9). Thus, the present data are not exactly comparable either temporally or in terms of absolute values but the fundamental relation between liver regeneration and the parathyroprivic state has not changed.

Results. The fundamental observation that parathyroidectomy (PTX) or thyroparathyroidectomy (TPTX) can seriously reduce the regenerative surge of hepatocyte DNA synthesis and mitosis following partial hepatectomy, has been confirmed (Figs. 1 and 2). Previously (4, 9), this effect was observed and studied only in animals which had undergone the PTX or TPTX operation 20–24 hr before partial hepatectomy. However, it has been found subsequently that the size of the liver's regenerative response depends on the length of the interval between removal of the glands and the partial hepatectomy. Thus, when the interval was only 12 hr, the regenerative response was essentially normal despite the absence of the parathyroid and thyroid glands (Figs. 1A and 1B). On the other hand, the lowering of the regenerative response by parathyroidectomy or thyroparathyroidectomy became evident only when longer intervals (24–72 hr) were used (Figs. 1A, 1B and 2).

When the liver resection was performed 24 hr after removal of the glands, the proportion of hepatocytes synthesizing DNA at 26 hours after partial hepatectomy was much lower (50%–60%) than the proportion of DNA-synthesizing cells at 26 hr in either control, partially hepatectomized, animals, or in animals partially hepatectomized 12 hr after parathyroidectomy or thyroparathyroidectomy (Fig. 1A). The cellular potential to initiate DNA synthesis continued to decline with time after the PTX or TPTX operation. Thus, only 15% of the normal DNA-synthetic response could be evoked when the time between parathyroid removal and the liver resection was lengthened to 72 hr (Fig. 1A).

The fraction of proliferatively activated

liver parenchymal cells which were in mitosis 28 hr after the liver resection also declined as the length of the interval between parathyroidectomy or thyroparathyroidectomy and the partial hepatectomy was increased beyond 12 hr (Fig. 1B). When the interval between the operations was 24 hr, the mitotic activity dropped to about 30% of the control level. A further decline to about 10% of the control level occurred when the interval was lengthened to 72 hr (Fig. 1B). This decrease in mitotic activity, particularly between the 12 and 24 hr intervals, appeared to be greater than anticipated from the proportion of cells synthesizing DNA (cf. Fig. 1A). The meaning of this apparent disparity is not known nor have such differences been observed previously in aparathyroid animals (9).

In the foregoing experiments, the DNA-synthetic and mitotic activities were measured at only single times after partial hepatectomy (26 and 28 hrs for DNA and mitosis, respectively) which were at or near the current peak period of these responses in the normal, partially hepatectomized, animals. To ensure that the apparent failure of partial hepatectomy to fully activate proliferation in the liver remnant of PTX or TPTX animals was not simply due to a delay in the initiation of DNA synthesis, we measured the flow of hepatocytes into the DNA-synthetic (or S) phase for 50 hr after partial hepatectomy in animals which had been either parathyroidectomized or thyroparathyroidectomized 72 hr previously. Although there was a slow increase in the percentage of DNA-synthesizing parenchymal cells in the PTX and TPTX rats, which commenced between 18 and 22 hrs and reached its maximum at 30 hr after partial hepatectomy, it was evident that there was a large, and permanent, loss of proliferative capacity of hepatocytes in the PTX and TPTX animals (Fig. 2).

Discussion. Unknown factors liberated by partial hepatectomy proliferatively activate hepatocytes. Deoxyribonucleic acid (DNA) synthesis and mitosis are initiated hours later in these activated cells. The present study indicates that the parathyroid hormone is not involved in the initial activation process but does control the subsequent initiation of DNA synthesis. This conclusion is based on the observations that hepatocyte proliferation declines when the partial hepatectomy is carried out 24 or more hours after removal of the parathyroid glands (Figs. 1 and 2) but when partial hepatectomy is carried out 12 hours after removal of the glands, the DNA synthesis and cell proliferation is completely normal (Fig. 1). Under this latter parathyroprivic condition, proliferation occurred despite the absence of parathyroid hormone in the circulation (10, 11, 14) and despite the fact that partial hepatectomy could not have induced a hormonal surge (see Introduction). A surge of hormone would be essential if parathyroid hormone were a factor in the initial activation (as opposed to initiation of DNA synthesis), otherwise the level of circulating hormone in the normal, intact, animal would be expected to keep the parenchymal cells of the intact liver in a constant state of active proliferation, which is obviously not true. It is conceivable that parathyroid hormone could remain attached to the receptors on the liver cells for 12 hr and thus maintain the competence of the already activated cells to enter DNA synthesis and mitosis. However, such adsorbed hormone likewise cannot be involved in the initial activation process because the maximum amount of hormone adsorbed to the proliferating hepatocytes in the parathyroprivic animals cannot be greater than the amount of hormone adsorbed to the nonproliferating (nonactivated) liver parenchymal cells in the normal intact animal. Thus, it appears that circulating or adsorbed parathyroid hormone is not a factor in the proliferative activation process induced by partial hepatectomy but is needed to keep the liver cells in a "state of readiness" which permits them to ultimately initiate DNA synthesis and mitosis after activation. This hormonally induced, competent state takes one or more days to deteriorate in the absence of hormone.

It is not yet possible to decide whether the decline in the ability of liver cells to initiate the proliferative process is due to the absence of hormone or the associated

hypocalcemia. The hypocalcemic, aparathyroid condition, in addition to decreasing the capacity of hepatocytes to regenerate, also inhibits the proliferation of the continuously "cycling" precursor cells in thymus and bone marrow, causing severe hypoplasias of both tissues (5–8, 13). Available evidence indicates that parathyroid hormone controls the initiation of DNA synthesis in the already proliferatively activated thymic lymphoblasts, probably by affecting membrane calcium permeability and increasing the cell's sensitivity to stimulation by the extracellular calcium ion at a point late in the G1 phase of the growth-division cycle (12, 13). In these lymphoblasts, as well as in chicken fibroblasts cultivated *in vitro,* extracellular calcium ions trigger a "master reaction" which starts the several processes leading to growth, DNA synthesis and ultimately cell division (13). From these considerations, we might speculate that the parathyroid hormone continuously maintains the liver cell membrane in a state which would allow extracellular calcium ions to trigger such a "master reaction" when the activated cells reach a specific point in the early phases of their posthepatectomy development. Thus, impairment of this hormone-induced, calcium-sensitive, state by removal of the parathyroid glands, would prevent the proliferatively activated liver parenchymal cells from starting their replicative processes.

Summary. Removal of the parathyroid glands in rats reduced the initiation of DNA synthesis and proliferation which normally occurs in the liver parenchymal cells remaining after partial hepatectomy. However, this effect was observed only when the parathyroid glands were removed 24 or more hours before partial hepatectomy. When the glands were removed 12 hr before the partial hepatectomy, there was no decrease in the ability of hepatocytes to initiate the regenerative process. These observations suggest that parathyroid hormone is needed to maintain the competence of liver parenchymal cells to proliferate when activated by partial hepatectomy, but is not involved in the initial activation process itself.

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