Parathyroid Hormone: A Possible Initiator of Liver Regeneration[1] (36723)

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(Introduced by Helen J. Morton)
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The liver tissue remaining after subtotal hepatectomy in mammals undergoes a striking compensatory hyperplasia (1, 2, 18, 21). The initiator (or initiators) of the chain of events leading to this proliferation of liver cells has escaped detection, but available evidence indicates that it may be of humoral origin (2, 3, 7, 18). The strongly mitogenic parathyroid hormone might be such a humoral initiator, since it is a principal stimulatory regulator of cell proliferation in the bone marrow and thymus gland (9, 10, 14, 16, 22, 23).

In the present communication, some support for a role of the parathyroid hormone in liver regeneration is provided by the observation that removal of the parathyroid glands considerably delays and reduces the initiation of DNA (deoxyribonucleic acid) synthesis and mitosis which is normally induced in liver parenchymal cells by partial hepatectomy. Further support for parathyroid hormone involvement is shown by the fact that partial hepatectomy produces the physiological "trigger" for the endogenous release of parathyroid hormone, namely, a very early, transient, hypocalcemia.

Materials and Methods. Parathyroidectomy (PTX) was performed on specific-pathogen-free, male (190–230 g), Sprague-Dawley rats. The animals were anesthetized with Fluothane, their thyroid–parathyroid complex exposed and the parathyroid glands destroyed by electrocautery (5, 14). Sham-PTX control animals were subjected to exactly the same surgical manipulations, but the glands were not destroyed. Any PTX animal which had a total calcium concentration of 7 mg/100 ml of plasma at the time of sacrifice was considered to have been an operative failure and was not used.

Twenty to 24 hr after the PTX or sham-PTX operations, when all circulating parathyroid hormone in the PTX animal would have disappeared, the median and left lateral lobes (68% of the mass) of the liver were removed (under aseptic conditions) from these animals according to Higgins and Anderson (4). Sham hepatectomies (laparotomies) were performed in exactly the same manner except for the final ligation and removal of the two lobes.

Since the PTX and partially hepatectomized animals considerably, but variably, reduced their food intake [cf. (16)], they were not fed during the experimental period which commenced at the time of the first operation. Distilled water was provided *ad libitum*.

At various times after these operations, the animals were anaesthetized with ether and 4 to 5 ml of blood were removed from the abdominal aorta for calcium determinations. When both the ionic and total calcium concentrations were to be measured, the blood was withdrawn in plastic syringes with siliconized hypodermic needles and then placed in siliconized tubes under paraffin oil for centrifugation; the layer of paraffin oil prevented changes in the ionized calcium concentration which would be caused by an escape of carbon dioxide (19). When only the total plasma calcium concentration was to be measured, the blood was withdrawn with syringes moistened with heparinized saline. The ionic calcium concentration in the plasma was determined (within 2 hr of blood withdrawal) using an Orion calcium "flow-through" electrode system (model 99-20, Orion Research Inc., Cambridge, MA). The total calcium concentration was determined

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by titration with EGTA (ethylene bis (oxyethylenenitrilo) tetraacetic acid) in a Fiske automatic fluorometric titrator (Fiske Assoc. Inc., Uxbridge, MA) using calcein as the indicator.

The right lateral lobe of the liver was also removed for autoradiography and histology immediately after withdrawal of the blood. The mitotic activity of the parenchymal cells was determined by fixing small blocks of the tissue in a solution of 10% formalin and 2% acetic acid and staining the cells in 5 μ sections with hematoxylin and eosin. These sections were scored for the proportion of the parenchymal cell nuclei which were in prophase, metaphase, anaphase and telophase (mitotic index). A minimum of 2000 and a maximum of 10,000 nuclei were examined in each liver sample.

To determine the proportion of parenchymal cells synthesizing DNA, autoradiographs were prepared from the right lateral lobe of livers of rats which had been injected with ^{3}H-thymidine (1.0 μCi/g of body weight; sp act 20 Ci/mmole; New England Nuclear Corp., Boston) 1 hr before sacrifice. The tissue was fixed in formol–acetic acid, embedded in paraffin, sectioned at 3 μ, dewaxed and washed three times in a solution of 10 m*M* unlabeled thymidine in distilled water. The sections were covered with nuclear track emulsion NTB-2 (Eastman Kodak, Rochester) and stored at 4° for 14 days. The autoradiographs were then developed and the underlying cells stained with hematoxylin and eosin. For each liver sample, 2000 to 10,000 parenchymal cell nuclei were examined to obtain the ^{3}H-thymidine labeling index.

Results. Eighteen hours after partial hepatectomy, the parenchymal cells in the residual hepatic tissue of control (sham-PTX) animals began to incorporate ^{3}H-thymidine, and the proportion of cells synthesizing DNA then quickly rose to a peak value of 32.6% at 22 hr (Fig. 1A). In PTX animals, on the other hand, where no circulating parathyroid hormone is present, the proportion of cells which incorporated ^{3}H-thymidine rose much more slowly and reached a maximum value of only 15% at 36 hr after partial hepatectomy (Fig. 1A).

In both the sham-PTX and PTX animals, the stimulated parenchymal cells began to enter mitosis 4 to 6 hr after they had started synthesizing DNA (Fig. 1). In the control (sham-PTX) animals, the fraction of mitotic parenchymal cells then sharply increased to a maximum value of 4.0% at 28 hr after partial hepatectomy (Fig. 1B). However, the mitotic index in the remaining liver tissue of PTX animals reached a peak value of only 2.0% at 40 hr after partial hepatectomy (Fig. 1B).

The proliferative response of the control (sham-PTX) animals to partial hepatectomy

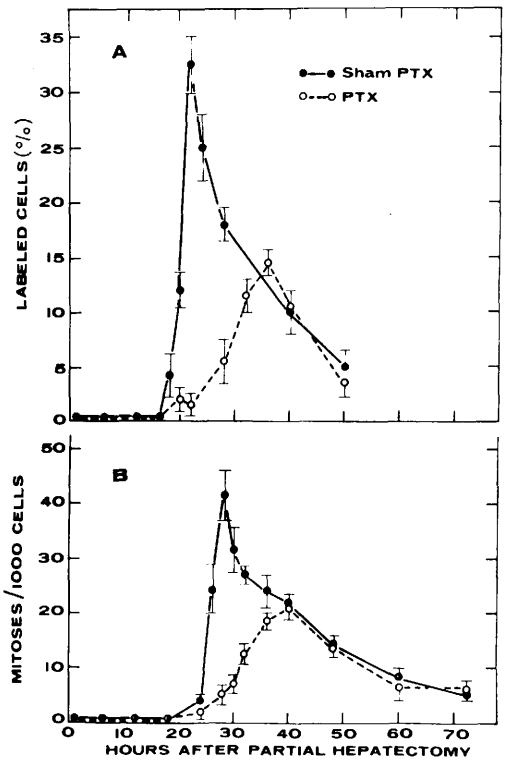

FIG. 1. The effect of parathyroidectomy (PTX) on the proportion of parenchymal liver cells which incorporated ^{3}H-thymidine (A) and entered mitosis (B) at various times after partial hepatectomy. The PTX and the control sham-PTX operations were carried out 20 to 24 hr before the partial hepatectomies. Food was denied during these experiments commencing at the time of the PTX or sham-PTX operation. The ^{3}H-thymidine (1 μCi/g) was injected intraperitoneally 1 hr before sacrifice. Each point and vertical bar is the mean value and SEM from 5 to 9 rats.

in the present study is very similar to those reported for rats by other investigators (2, 12, 20, 21). As expected, laparotomies (sham hepatectomies) carried out during the present investigation failed to induce any increase in either ^{3}H-thymidine incorporation or mitotic activity; the proportion of cells incorporating ^{3}H-thymidine did not exceed 0.05% and the proportion of cells in mitosis did not exceed 0.01%.

In these experiments, the total calcium level in the blood plasma was routinely measured at the time of sacrifice to establish that parathyroidectomy had successfully produced a pronounced hypocalcemia. During the course of these routine analyses, it became apparent that partial hepatectomy itself caused an early hypocalcemia (Fig. 2). In sham-PTX animals, subtotal hepatectomy was followed by a significant ($p < .001$) and sharp drop in the blood calcium level by 6 hr with a partial recovery by 12 hr (Fig. 2). Moreover, in the already hypocalcemic PTX animals, hepatectomy caused an even further, and prolonged, decline in the blood calcium level (Fig. 2). The original hypocalcemic level was not regained in these animals until 30 to 40 hr after the partial hepatectomy.

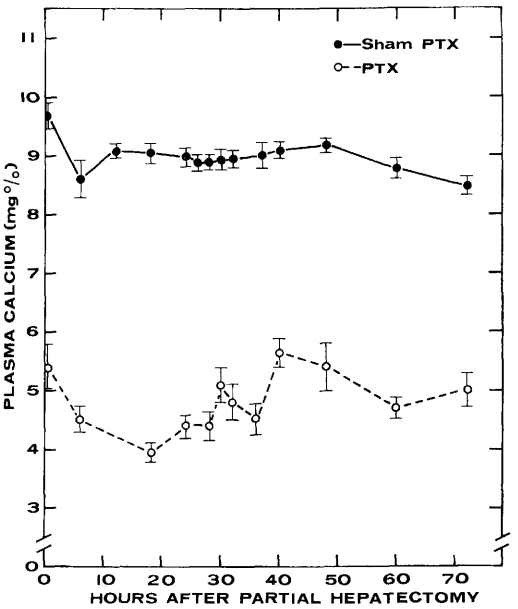

FIG. 2. The level of total calcium in the plasma of parathyroidectomized (PTX) or sham-PTX control rats at various times after partial hepatectomy. The PTX and the sham-PTX operations were carried out 20 to 24 hr before the partial hepatectomy was performed. Food was denied commencing at the time of the PTX or sham-PTX operation. Each point and vertical bar represents the mean value and SEM from 5 to 9 rats.

Since parathyroid hormone was evidently involved in the posthepatectomy initiation of liver cell proliferation, and since it was a well-established fact that hypocalcemia is the physiological signal for the secretion of endogenous parathyroid hormone (6, 13, 17), we examined the hypocalcemic effect of subtotal hepatectomy in more detail. In normal (not sham PTX) animals, the operation caused a significant transient lowering of both the total and ionic calcium concentrations. The total calcium concentration began to fall during the first hour after the operation and it reached its lowest value at 6 hr (Fig. 3A). Between 6 and 24 hr, the calcium level slowly returned to normal (Fig. 3A). Only a slight hypocalcemia developed in sham-hepatectomized (laparotomized) animals (Fig. 3A).

The decline in the physiologically active ionic calcium level in the plasma was proportionally the same as the decline in the total calcium concentration. The ionic calcium concentration dropped from 5.6 to 5.0 mg/100 ml ($p < .001$) between 4 and 6 hr after partial hepatectomy (Fig. 3B). Normal levels were not regained until 18 hr. No changes occurred in the ionic concentration in the plasma of control, laparotomized animals (Fig. 3B).

Discussion. The present observations show that the normal proliferative response of liver parenchymal cells to partial hepatectomy requires the presence of the parathyroid glands and is preceded by a transient hypocalcemia which is known to be the physiological signal for the release of endogenous parathyroid hormone (6, 13, 17). When a hypocalcemia is induced by partial hepatectomy in the absence of the parathyroid glands, there is a delayed and considerably reduced increase in the number of cells which initiate DNA synthesis and enter mitosis. This suggests that a hypocalcemia-induced increase of parathy-

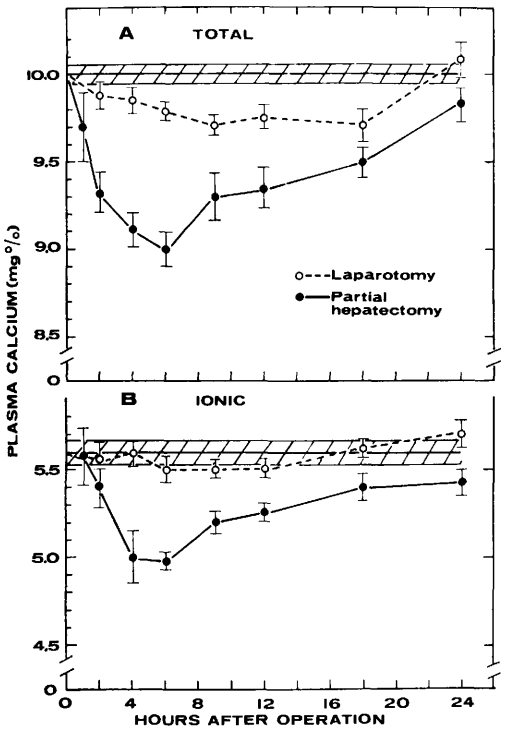

FIG. 3. The total (A) and the ionic (B) calcium concentration in the plasma of partially hepatectomized and laparotomized rats. Food was denied during the observation period. Each point and vertical bar is the mean value and SEM from 7 to 14 rats. The horizontal lines and hatched areas are the mean values ± SEM from 44 normal rats examined throughout the experimental series. The total calcium values from 2 to 20 hr and the ionic values from 4 to 12 hr are statistically significant ($p < .001$) from those in normal rats.

roid hormone may be one of the humoral events which is responsible for the initiation of liver regeneration.

The fact that the initiation of DNA synthesis and mitosis in liver are impaired in parathyroidectomized rats, where a persistent and profound hypocalcemia prevails as well as a lack of parathyroid hormone, could imply that part of the liver regeneration is mediated by the hormone or by calcium. It is known that calcium can stimulate the proliferation of cells in the thymus and bone marrow but that an *increase* in the extracellular level of calcium above normal is always needed to implement this effect (9, 10, 14, 16). Since the concentration of plasma calcium never exceeded the normal levels in the parathyroid-intact, partially hepatectomized animals, it would seem unlikely that calcium alone is an initiator of liver regeneration.

Although a direct role of parathyroid hormone is yet to be proven, it is important to note that an artificial hypocalcemia induced by the injection of sodium caseinate causes a parathyroid hormone-induced stimulation of cell proliferation in bone marrow (15). This may also be the case for the cell proliferation induced by other calcium chelators, *i.e.*, ethylenediaminetetraacetic acid and organic phosphate (11). In addition, a severe hemorrhage, specifically the loss of red blood cells, is known to induce a hypocalcemia which stimulates the proliferation of bone marrow only when parathyroid hormone is available (8). It would now appear that there are several situations, two involving a loss of tissue (hemorrhage and partial hepatectomy), in which a hypocalcemia, in the presence of functional parathyroid glands, precedes the initiation of cell proliferation.

Summary. The stimulation of DNA synthesis and mitotic activity in liver cells after partial hepatectomy is reduced to about 50% of their normal value and delayed 12 to 14 hr in parathyroidectomized rats. It has also been observed that partial hepatectomy, itself, induces an early, transient, hypocalcemia which precedes the initiation of DNA synthesis and cell proliferation. These observations suggest that the parathyroid hormone might be a humoral agent involved in the initiation of hepatic regeneration.

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