Stimulation of Growth by Partial Hepatectomy.* (24876)

K. E. PASCHKIS,† J. GODDARD, A. CANTAROW‡ AND S. ADIBI§
Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia

We have previously reported that growth of certain transplanted rat tumors is enhanced by partial hepatectomy as compared with growth of these tumors in sham-operated controls(1). Previously, 3 groups of investigators had shown that partial hepatectomy in one of a pair of parabiotic rats induced increased mitoses in the liver of the other, intact, parabiont(2,3,4). Because these observations indicated enhancement of certain growth processes in the presence of regenerating liver, the effect of the latter was investigated further. Studies were made of the effect of partial hepatectomy and liver regeneration on: (a) mitotic rate in cornea and skin; (b) compensatory hypertrophy of kidney following unilateral nephrectomy; (c) epiphyseal width in the hypophysectomized rat.

Material and methods. Partial hepatectomy was performed under ether anesthesia by the method of Higgins and Anderson whereby 67% of liver is removed(5). Male rats of Wistar strain, obtained from Barkbridge Farms, N. J., were used, except in the study of epiphyseal width. The latter experiments were performed on hypophysectomized female rats of Sprague Dawley strain, purchased from Hormone Assay Labs, Chicago. Hypophysectomy was performed at the age of 26 days, and the experiments 2 weeks later. *Corneal mitosis.* Partially hepatectomized and control rats were killed by exsanguination under ether anesthesia on fourth day after operation; all animals received an injection of colchicine (1 mg/kg body wt) 8 hours prior to death. Control rats were subjected to "sham operation" on day on which the experimental group was partially hepatectomized. The sham operation consisted of laparotomy under ether anesthesia; liver lobes were pushed out of abdomen through the incision, then returned to abdominal cavity. At autopsy, the eyes were fixed in Bouin-Hollande solution; after fixation the cornea was dissected out, embedded in paraffin, and sectioned serially at 6 μ. Sections were stained with Mallory's acid-hematoxylin, or with Masson's iron-hematoxylin. 12-14 sections on either side of the equator were used for mitosis counts. Randomization of microscopic fields was performed by use of random numbers of Kendall and Smith(6). To avoid counting mitoses twice, only alternate sections were used. The mitotic index was calculated by the formula $\frac{\text{mitotic nuclei}}{\text{quiescent nuclei}} \times 100$. The Rank test, according to Kruskal and Wallis(7) was applied to evaluate significance (p). *Compensatory hypertrophy of kidney.* The experimental group was subjected to partial hepatectomy; at the same time one kidney was removed. In controls, only unilateral nephrectomy was performed. Weight of extirpated kidney and body weight were recorded. Three weeks later all animals were killed by exsanguination under ether anesthesia and right kidneys were weighed. The difference between weights of the 2 kidneys is a measure of compensatory hypertrophy following unilateral nephrectomy. The significance of difference of mean increments in kidney weight between control group (nephrectomy only) and nephrectomized hepatectomized group was determined by Fisher-"Student" test. *Epiphyseal growth in hypophysectomized rats.* Female rats hypophysectomized on 26th day of life were subjected to partial hepatectomy 2 weeks after hypophysectomy; hypophysectomized controls were subjected to sham operation as described above. In the first 2 series postoperative mortality was so high that no definite conclusion could be drawn from the few survivors. Thereafter, both hepatectomized and sham-operated animals were given 2.5 mg cortisone acetate by subcutaneous injection daily

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† Dept. of Physiology and of Medicine.
‡ Dept. of Biochemistry.
§ Student assistant, oncologic teaching grant of Nat. Cancer Inst.

TABLE I. Mitoses in Cornea following Partial Hepatectomy.

	No. of animals	Median mitotic index
Controls	7	.722
Hepatect.	11	2.080
		$P_{(H)} < .001$*

* Rank Test (Kruskal and Wallis).

beginning on day of operation. All animals were killed on 6th day after operation. The tibiae were fixed in Bouin-trichloracetic acid, and decalcified in a solution containing 5% trichloracetic acid and 10% formaldehyde. After embedding in paraffin, serial sections were made of the region of proximal epiphysis and were stained with hematoxylin eosin. Measurements of epiphyseal width were made on central sections, with use of eye-piece micrometer(8). For statistical evaluation, the p of difference between the 2 groups was determined by Fisher's "Student" method.

Results. Mitosis stimulation, cornea and skin (Pinna). Four days after partial hepatectomy, the mitotic index in the cornea was significantly higher than in cornea of sham-operated controls (Table I). No increase of mitotic activity was found in the skin (pinna). (Not tabulated).

Compensatory enlargement of kidney. In rats subjected simultaneously to unilateral nephrectomy and partial hepatectomy, compensatory enlargement of remaining kidney was greater than in controls, subjected only to unilateral nephrectomy. The difference was statistically significant (Table II).

Growth of the tibial epiphysis in hypophysectomized rats. Six days after partial hepatectomy there was a highly significant increase in epiphyseal width as compared with that in sham-operated controls (Table III). The possible growth inhibiting effect of the administered cortisone acetate we consider of minor importance because both hepatectomized and control rats received identical treatment.

Discussion. Experiments here reported show stimulation of growth of 3 different tissues following partial hepatectomy. These 3 tissues differ widely in their biological characteristics. Intensive and rapid growth induced in liver remnant following partial hepatectomy has long been known. Occurrence of a mitotic reaction in liver of a normal, intact parabiont, following partial hepatectomy in the other, first demonstrated that a growth stimulus was carried humorally following partial hepatectomy(2,3,4). These data could be interpreted as indicating presence of an organ specific growth-stimulating agent. Our previously reported findings of increased tumor growth in the presence of regenerating liver(1) and extension of this effect in the present report, to non-tumor tissues, indicate that the growth stimulus is not organ specific.

TABLE III. Effect of Partial Hepatectomy on Epiphyseal Width of Proximal Tibia of Hypophysectomized Rats.

	No. of animals	Mean epiphyseal width
Sham operated controls	17	212.0 ± 4.67*
Hepatectomized	10	246.7 ± 6.74
		$P_{(t)} < .001$†

* Stand. error of mean.
† T-test (Fisher's Student method).

The question whether such growth stimulation is due to an active growth stimulating agent circulating in partially hepatectomized

TABLE II. Effect of Partial Hepatectomy on Compensatory Hypertrophy of Kidney.

	No. of animals	Mean incr. in kidney wt (g) Absolute	Relative*	Body wt incr. (g)
Control nephrect.	18	.320 ± .03†		57.9
Nephrect., hepatect.	8	.430 ± .04		50.5
$P_{(t)}$ of diff.‡		.05		
Control nephrect.	9	.321 ± .02	.080 ± .01	40 ± 14.2
Nephrect., hepatect.	7	.544 ± .07	.228 ± .05	55.4 ± 28.2
$P_{(t)}$ of diff.		<.01	<.01	<.2

* Wt of each kidney recorded in % of body wt at time kidney was obtained.
† Stand. error of mean.
‡ T-test (Fisher's "Student" method).

rats, possibly secreted by the regenerating liver, or whether it is due to removal of a growth inhibitor by partial hepatectomy, was first posed by Bucher *et al.*(2). Glinos has recently reviewed the evidence for the latter mechanism, implicating plasma proteins as the regulating factor, or the agent of "chemical communication"(9). On the other hand, Friedrich-Freksa and Zaki have found that injection of serum of hepatectomized, but not of intact rats into normal recipients induces mitoses in the liver. This is interpreted as evidence for presence of a growth-stimulating factor(10). Glinos discounts this report because he was unable to confirm it; however Stich and Florian have confirmed, in part, the findings of Friedrich-Freksa, inasmuch as they found that injection of serum from partially hepatectomized rats increased the mitotic count of regenerating liver in the recipient animal. Injection of serum from intact donors, or of homogenates of normal liver, inhibited mitosis in the regenerating liver. The authors also interpret their results as indicating regulation of liver growth by an inhibitor present in normal liver, and evidently in circulating blood(11). However the fact that serum from hepatectomized donors showed not merely absence of inhibiting action, but actual stimulation, suggests the possibility of a balance between stimulating and inhibiting factors(12). The studies cited above were concerned with regulation of liver regeneration and were interpreted in terms of organ-specific growth regulators. It is not known whether the non-organ-specific growth stimulation incident to liver regeneration here described, is subject to similar inhibitory and regulatory factors; this possibility is currently being investigated in our laboratories.

Summary. It has been shown by others that partial hepatectomy in one partner of parabiotic rats induced mitoses in liver of the other, intact parabiont. We found previously that growth of transplanted tumors was enhanced by partial hepatectomy of the host. In this paper stimulation of non-malignant growth following partial hepatectomy in the rat is reported. Mitotic rate in cornea, and compensatory hypertrophy of kidney is increased. In hypophysectomized rats partial hepatectomy is followed by increased width of tibial epiphysis. These findings are discussed with regard to organ specificity and mechanisms of growth regulation.

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