REGENERATION OF THE LIVER

Absence of a "Humoral Factor" Affecting Hepatic Regeneration in Parabiotic Rats

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The mechanism by which regeneration of the liver is initiated and controlled has long been an important but unsolved problem in biology and medicine. Three facets into which hepatic regeneration might be subdivided for purposes of investigation are: (a) its initiation following partial hepatectomy or injury; (b) its modification, once begun, as by hormones, nutrients, and possibly blood flow; and (c) its termination when the organ has regained its original size or function. The present work is concerned primarily with elucidating the factor or factors that initiate the regenerative process, although all 3 aspects are probably closely interrelated.

The reports by Christensen and Jacobsen,[1] Bucher, Scott and Aub,[2] and Wenneker and Sussman[3] of a stimulus to mitosis in the liver of one parabiotic rat as the result of partial hepatectomy in its partner directed attention to the possibility that hepatic regeneration might be controlled by an alteration in the composition of the blood, a so-called "humoral factor." Many studies of the control of regeneration followed this work. These included the injection of serum and liver homogenates from partially hepatectomized rats into normal rats, measurements of chemical constituents of the blood, effects of serum on tissue culture growth of cells, and investigation of the organ specificity of the stimulus to hepatic regeneration. As reviewed elsewhere,[4] the findings and conclusions in these investigations were conflicting. Consequently, the evidence that a humoral factor is involved in regeneration of the liver rests upon the original observations in parabiotic rats.

The 3 studies in parabiosis cited indicated that a stimulus to hepatic regeneration was detected in a "nonoperated" † partner by quantitation

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† The term "nonoperated" in referring to parabiotic rats is used to mean that no operative experimental procedure was done after parabiotic union was established.

of mitoses in its liver. In none of the 3 studies was there an indication that cross circulation between the parabiotic animals had been demonstrated, although it may be assumed that it was present. Christensen and Jacobsen used 3 sets of parabiotic paired rats, without parabiotic controls; they found hepatic mitoses to be slightly more numerous in the 3 nonoperated partners than in single nonoperated rats.[1] Wenneker and Sussman used 8 pairs of parabiotic rats without either nonoperated or sham operated control parabiotic animals.[3] They sacrificed 1 or 2 pairs at 2, 3, 5, 8 and 14 days after partial hepatectomy in one member. Counts of hepatic mitoses in the nonoperated members were compared with those in nonoperated single rats and were found to be slightly greater at 2 to 8 days after operation.

Bucher and co-workers used 22 pairs of parabiotic rats in 3 experiments at 24, 48 and 72 hours after partial hepatectomy and 3 sets of "triplet" parabiotics at 48 hours after partial hepatectomy.[2] As controls, mitosis counts were made in the liver portions removed at operation; no separate nonoperated or sham operated parabiotic rats were studied. In one of the experiments using paired rats, the difference between the number of hepatic cell mitoses in the nonoperated partners and in the control livers was not of statistical significance when all rats were used for calculations. The authors did not use some rats for statistical computation because on histologic examination cross circulation was not considered to have been established. In the other 2 groups the differences were of statistical significance. Colchicine enhanced none of the differences. In the parabiotic triplets, partial hepatectomy was performed in 2 members; here there was an even greater mitotic count in the liver of the nonoperated rat. Other indexes of regeneration investigated were the hepatic content of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), alkaline phosphatase, and total nitrogen, but these gave no evidence of a regenerative response.

A further study of parabiotic rats, carried out since the above reports, failed to confirm the earlier results. Islami, Pack and Hubbard[5] used 5 parabiotic pairs, with the liver removed at operation as the control. Their data showed no evidence, as indicated by mitoses, of a stimulus to regeneration in the liver of the nonoperated partner. It was suggested, however, that there was a depression of the regenerative response in partially hepatectomized parabiotic rats as the result of union with nonoperated rats.

The present investigation was undertaken after studies in which plasma from cirrhotic rats, rats with fatty liver, and those that had undergone partial hepatectomy was infused into normal rats.[4] The purpose of the preliminary investigations was to determine whether the

stimulus to increased mitosis and DNA synthesis observed in the livers of the 3 groups of donor rats [6] could be transmitted to the recipients. It was assumed from published reports that there would be a humoral stimulus to regeneration in the partially hepatectomized rats. No response in DNA synthesis or mitosis was found in any of the recipients, however. This was the case in animals that received plasma from partially hepatectomized rats. Moreover, the infusion of plasma from normal rats into animals that had undergone partial hepatectomy failed to inhibit regeneration. Therefore, it was considered necessary to restudy the existence of a humoral factor affecting regeneration of the liver in parabiotic rats.

Material and Methods

A total of 494 male Sprague-Dawley and 64 male inbred rats were used in these experiments. They were housed in an air-conditioned room and fed Purina Lab Chow and tap water *ad libitum*. Sprague-Dawley rats were purchased from the Charles River Breeding Laboratory, Boston, at 21 days of age, and inbred rats of the WR strain were purchased from the Endocrine Laboratories, Madison, Wisconsin, as weanlings. Both strains were maintained in this laboratory until used.

Sprague-Dawley rats (214; 107 pairs) were joined in parabiotic union by the open coelomic method [7]; 42 (21 pairs) were joined by the closed coelomic method.[8] Sprague-Dawley rats (174; 58 sets) and 57 inbred rats (19 sets) were joined as "triplets" by the open coelomic method. The skin incision extended from shoulder to hip joint; the peritoneal incision extended from the rib cage caudally for about 3 cm. Adjacent fore and hind limbs of partners were fixed by placing wire sutures through the proximal bones. The ages at the time of union varied from 36 to 78 days, and all the Sprague-Dawley parabiotic rats except some of the group of pairs examined at 72 hours after operation were composed of litter mates. The latter group contained a few non-litter mates; this had no detectable effect on the parabiosis or the experimental results. In addition, when single rats were used as controls, the single rats were litter mates of the parabiotic animals whenever possible, or were from the same lot.

The presence of cross circulation was determined by prompt (within 20 minutes), grossly visible excretion of phenolsulfonphthalein dye (PSP) in the urine of both members of pairs after injection of 0.5 ml. of the dye intramuscularly into one partner, or its excretion in the urine of all 3 members of triplets after injection of 1 ml. into one partner. Before the experimental procedures were performed, 6 pairs and 1 set of triplets that failed to show cross circulation and 7 pairs and 2 sets of triplets that appeared sick or runted were discarded. Mortality following parabiotic union and before use in experiments was 39 per cent among the pairs, 16 per cent among Sprague-Dawley triplets, and 58 per cent among the inbred triplets. Most deaths occurred within 3 weeks after parabiotic union. After partial hepatectomy or sham operation, there was an additional mortality of 20 per cent in the pairs, 35 per cent among the Sprague-Dawley triplets, and 20 per cent among the inbred triplets. Death was not attributable to any specific cause, and occurred following both sham operation and partial hepatectomy. Two parabiotic triplet rats exhibited such extensive and diffuse intrahepatic inflammation that they were discarded. One was a nonoperated partner of partially hepatectomized rats and one had undergone sham operation. Exclusion of their mitotic and autoradiographic counts did not affect the statistical significance of the conclusions in their groups.

As the result of the mortality and the discard of unsuitable animals, the experi-

ments were carried out using 53 pairs, 31 sets of Sprague-Dawley triplets and 7 sets of inbred triplets. Studies were made 24, 48, 72, and 96 hours after partial hepatectomy or sham operation. Nonoperated parabiotic animals were included to determine the effect of parabiosis alone and to study biologic variations in hepatic mitosis and DNA synthesis. The duration of parabiosis in the animals used was 7 to 46 days. In addition to parabiotic rats, 60 single Sprague-Dawley rats and 6 inbred rats were used as controls; 28 of the Sprague-Dawley animals underwent partial hepatectomy, 27 underwent sham operation; and 5 Sprague-Dawley and 6 inbred rats were nonoperated controls.

Partial hepatectomy, in which 61 to 80 per cent of the liver was removed, was performed in a standard manner under ether anesthesia. A double ligature was placed around the anterior and left lateral lobes, followed by excision of these lobes.[9] Sham operation consisted of a laparotomy with manipulation of the liver. Four hours before sacrifice tritiated thymidine (H^3-thymidine) of specific activity 1.9 curies per mM was injected intraperitoneally in divided amounts within less than 10 minutes. The dose was one μc. per gm. of body weight in all rats except the triplets examined 24 hours after operation. These received a dose of 0.5 μc. per gm. of body weight. The animals were killed by ether and necropsied immediately; the liver was weighed and slices were fixed in 10 per cent neutral buffered formalin.

Paraffin sections cut at 6 to 10 μ were stained with hematoxylin and eosin for mitosis counts. Stripping film autoradiographs stained with hematoxylin were prepared as described elsewhere[10]; exposure time of the films was 4 weeks. Mitotic figures, from late prophase through anaphase, were counted in 50 consecutive fields at a magnification of 560 times. Mitotic figures were noted frequently in Kupffer cells in parabiotic rats. These were counted and were present to the same extent in all groups except for an expected increase following partial hepatectomy; thus they were not considered further.

Hepatic nuclei labeled with H^3-thymidine were counted in autoradiographs in 100 consecutive fields at a magnification of 560; fields containing large portal areas and large blood vessels were positioned so that the connective tissue, bile ducts and vessels were omitted. The number of hepatic cells in a field (560 $\times$) was determined by using an eyepiece grid marker. The results were expressed per 100,000 hepatic cells. In livers with no mitotic figures or H^3-thymidine-labeled cells in the fields counted, a total of 200 fields was examined before giving zero as the result.

In Text-figures 1 to 7 all rats indicated were of the Sprague-Dawley strain unless otherwise noted. Statements of statistical significance refer to the t test at the 5 per cent level of confidence.

Results

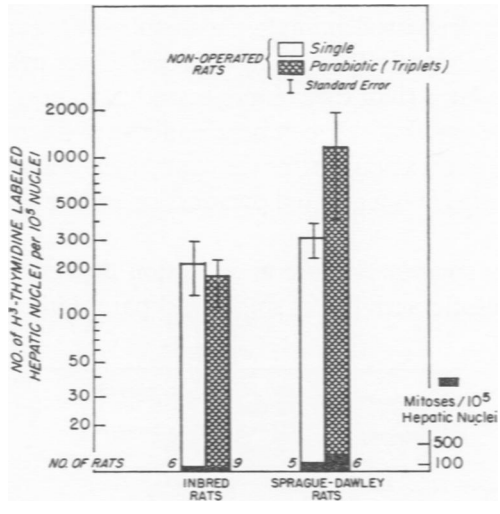
Histologic Features

The livers in both Sprague-Dawley and inbred parabiotic rats exhibited two findings which distinguished them from single animals. Plasma cells were present in the sinusoids as single cells or as clusters of 2 to 3 cells in 83 per cent of parabiotic rats, and small "granuloma-like" formations, composed of degenerating liver cells with an infiltrate of mononuclear cells and plasma cells occurred in 15 per cent. In addition, there were vascular lesions consisting of slight mononuclear cell infiltration in and around the walls of portal veins in two rats and in the adventitia of small hepatic arteries in a third. The livers were otherwise normal except in the two rats mentioned previously.

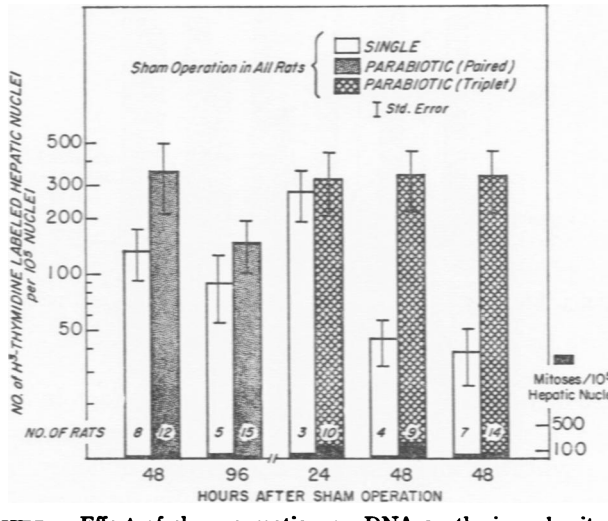
Autoradiographic and Mitosis Counts (Figs. 1 through 7)

In assessing other differences between parabiotic and nonparabiotic rats, it was observed that hepatic mitoses were more numerous and DNA was slightly greater in nonoperated parabiotic triplet rats than in nonoperated individual rats although the differences were not statistically significant (Text-fig. 1).

A difference in response to sham operation in parabiotic paired and



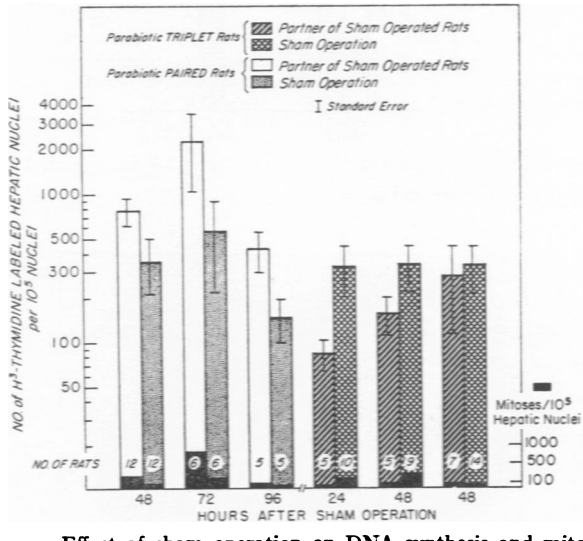
TEXT-FIGURE 1. DNA synthesis and mitosis in livers of nonoperated single compared with nonoperated parabiotic triplet rats.



TEXT-FIGURE 2. Effect of sham operation on DNA synthesis and mitosis in livers of single compared with parabiotic rats.

triplet rats as compared with individual rats was observed: hepatic DNA was consistently greater in parabiotic animals. In two separate experiments, studies were made 48 hours after a sham operation; triplet rats of the Sprague-Dawley strain showed significantly more DNA synthesis than their single sham operated controls. In one of the experiments mitotic activity was also significantly greater (Text-fig. 2). In addition, there was a variable response in different groups of animals. When individual rats were subjected to sham operation, their livers showed less DNA synthesis and mitotic figures 48 hours later than their nonoperated controls. In paired animals, the members that underwent sham operation had less hepatic DNA synthesis and fewer mitotic figures 48, 72, and 96 hours later than their nonoperated partners. In triplet parabiotic rats, where two lateral members underwent sham operation, the operated rats, 24 and 48 hours after the operations, showed no consistent differences from their nonoperated partners or from nonoperated sets of triplets.

These findings suggest that sham operation depressed hepatic DNA synthesis and mitotic activity in single and parabiotic paired rats, but

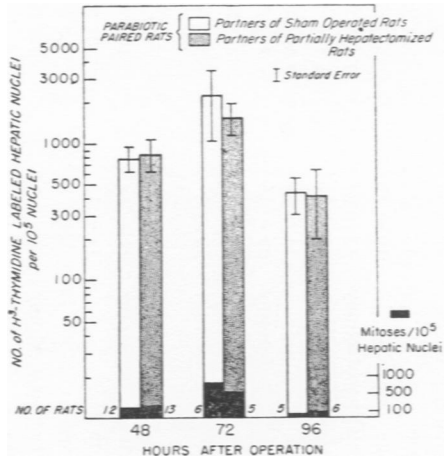


TEXT-FIGURE 3. Effect of sham operation on DNA synthesis and mitosis in livers of parabiotic rats.

did not do so in triplet rats. In addition to its effect in an operated parabiotic rat, a sham operation in one member may have stimulated hepatic DNA synthesis and mitotic division in the nonoperated partner. At 48 and 72 hours after operation, mitotic figures were significantly more numerous in a nonoperated rat than in its operated partner, and

more numerous, but not significantly so, than in parabiotic pairs where both rats were nonoperated. Hepatic DNA synthesis was similarly greater, but not significantly so, in the nonoperated partners. These findings were consistently observed in pairs, but were not noted in triplets (Text-fig. 3).

The effect of partial hepatectomy on hepatic DNA synthesis or



TEXT-FIGURE 4. Lack of effect of partial hepatectomy in one parabiotic rat on DNA synthesis and mitosis in liver of nonoperated partner.

mitotic activity in one member of paired rats was no greater than sham operation alone in the livers of nonoperated partners at 48, 72 and 96 hours (Text-fig. 4). Partial hepatectomy in 2 lateral members of triplets also caused no statistically significant elevation of hepatic DNA synthesis or mitosis in nonoperated partners 24 or 48 hours after operation. Variation in hepatic DNA synthesis and mitosis in the parabiotic rat was such that nonoperated Sprague-Dawley triplets showed higher average values than partners of operated rats (Text-fig. 5).

This would indicate that in neither paired nor triplet rats was there definite evidence of a humoral stimulus to hepatic regeneration (Table I). Sham operated inbred triplets were not included in these studies because their great mortality reduced the number available. From the observations in sham operated Sprague-Dawley triplets, hepatic DNA synthesis and mitosis would be expected to be the same as or greater than nonoperated controls.

Regeneration of the liver after partial hepatectomy in paired and triplet rats, although subject to variations in response, was similar to regeneration in single rats. There were no consistent differences at 24,

TABLE I

THE EFFECT OF PARTIAL HEPATECTOMY OR SHAM OPERATION IN PARABIOTIC RATS ON THEIR NONOPERATED PARTNERS

Hours between operation and sacrifice	No. of rats	Age at sacrifice (days)	Type of rat	Operation on parabiotic partners	Days between parabiosis and operation	H^3-thymidine labeled hepatic nuclei*	Hepatic mitoses*
Parabiotic pairs							
48	13	62 — 113	Sprague-Dawley	Partial hepatectomy	15 — 33	829 ± 219†	205 ± 68†
48	12	62 — 99	Sprague-Dawley	Sham	15 — 43	774 ± 162	192 ± 48
	7‡	78 — 113	Sprague-Dawley	None	19 — 46	313 ± 145	97 ± 22
72	5	61 — 103	Sprague-Dawley	Partial hepatectomy	15 — 31	1,551 ± 411	527 ± 126
72	6	54 — 94	Sprague-Dawley	Sham	10 — 28	2,244 ± 1,206	787 ± 194
	4	54 — 99	Sprague-Dawley	None	11 — 28	1,114 ± 516	558 ± 250
96	6	83 — 104	Sprague-Dawley	Partial hepatectomy	22 — 36	409 ± 213	95 ± 41
96	5	83 — 104	Sprague-Dawley	Sham	24 — 35	422 ± 130	60 ± 24
	7‡	78 — 113	Sprague-Dawley	None	19 — 46	313 ± 145	97 ± 22
Parabiotic triplets							
24	2	70	Sprague-Dawley	Partial hepatectomy	11 — 12	169 ± 99	16 ± 11
24	5	70	Sprague-Dawley	Sham	11 — 15	83 ± 20	32 ± 9
	3	70	Sprague-Dawley	None	11	92 ± 41	34 ± 14
48	5	75	Sprague-Dawley	Partial hepatectomy	8 — 21	818 ± 230	203 ± 110
48	7	75	Sprague-Dawley	Sham	7 — 22	281 ± 166	66 ± 26
	6	75	Sprague-Dawley	None	11 — 21	1,180 ± 766	291 ± 204
48	4	80 — 91	Inbred	Partial hepatectomy	10 — 17	1,053 ± 523	136 ± 61
	9	80 — 91	Inbred	None	14 — 18	166 ± 56	46 ± 18

* Expressed per 10^5 hepatic nuclei. H^3-thymidine labeled nuclei were counted in autoradiographs.

† Standard error.

‡ These represent the same rats; the 48 and 96 hour experiments were done simultaneously; one nonoperated group of controls was used for both.

TABLE II

REGENERATION OF THE LIVER FOLLOWING PARTIAL HEPATECTOMY IN PARABIOTIC AND SINGLE RATS*

Exper. no.	Hours after partial hepatectomy	No. of single rats	No. of parabiotic paired rats	No. of parabiotic triplet rats	Partial hepatectomy (%) †	H^3-thymidine labeled hepatic nuclei ‡	t test	Hepatic mitoses ‡	t test
1	48	4			76 ± 1§	10,060 ± 1,327§	$P < 0.05$	1,131 ± 157§	$P > 0.05$
			6		63 ± 4	5,508 ± 772		1,578 ± 288	
2	48	5			61 ± 4	7,596 ± 637	$P > 0.05$	1,283 ± 177	$P > 0.05$
			7		67 ± 3	5,946 ± 960		1,679 ± 319	
3	96	4			80 ± 2	1,596 ± 623	$P > 0.05$	284 ± 85	$P > 0.05$
			6		62 ± 7	1,863 ± 604		396 ± 161	
4	24	3			Not determined	31,539 ± 5,721	$P > 0.05$	5,279 ± 1,890	$P < 0.05$
				4		13,897 ± 6,305		402 ± 133	
5	48	8			73 ± 3	8,010 ± 1,145	$P > 0.05$	3,375 ± 445	$P < 0.01$
				11	65 ± 3	6,199 ± 669		1,837 ± 183	

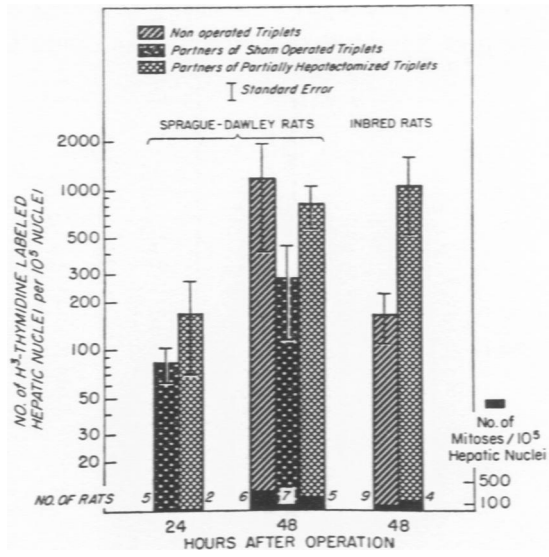
* A comparison with nonoperated parabiotic rats may be made in each experiment by referring to the appropriate group in Table I.

† Total liver weight at operation was calculated from body weight at sacrifice.

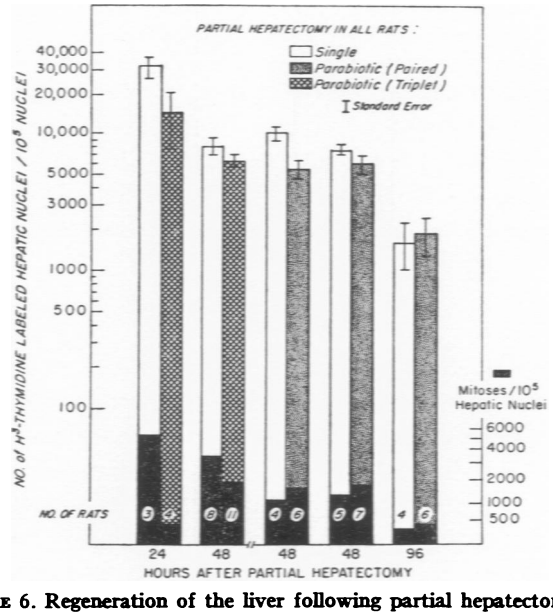
‡ Expressed per 10^5 hepatic nuclei. H^3-thymidine labeled nuclei were counted in autoradiographs.

§ Standard error.

48 and 96 hours (Text-fig. 6; Table II). The variation in response at any one time after partial hepatectomy was similar to that noted by us previously among groups of single rats.

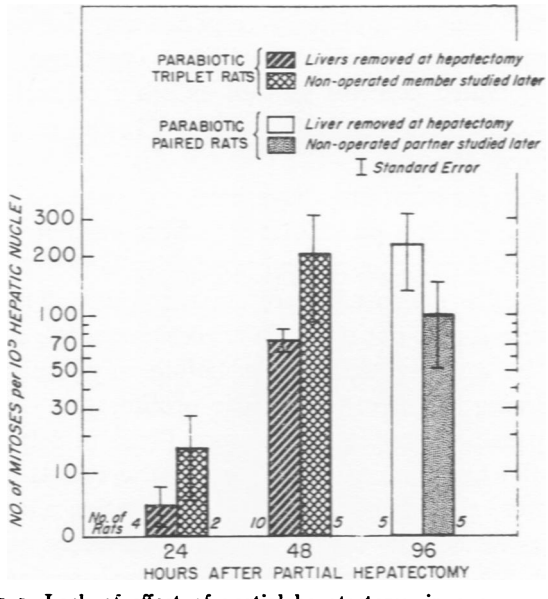


TEXT-FIGURE 5. Lack of effect of partial hepatectomies in 2 triplet parabiotic rats on hepatic DNA synthesis and mitosis in their nonoperated partners.



TEXT-FIGURE 6. Regeneration of the liver following partial hepatectomy in parabiotic and single rats.

Because other workers in parabiosis had done so, we compared mitotic activity in specimens removed at partial hepatectomy with that in the liver of nonoperated partners later, at time of sacrifice. The slightly increased amount of hepatic mitosis in the triplet rats whose partners had had partial hepatectomy was within the range of variation observed in these and other studies. The variation in mitosis is shown by comparing



TEXT-FIGURE 7. Lack of effect of partial hepatectomy in 1 or 2 parabiotic rats on hepatic mitosis in 1 nonoperated member.

the livers removed at partial hepatectomy in the 24 and 48 hour groups (Text-fig. 7). In the case of paired rats, livers obtained at sacrifice from nonoperated rats showed fewer mitoses than in the livers of their partners at the time partial hepatectomy had been done. Here also, evidence of a stimulatory humoral factor after partial hepatectomy was not manifest.

COMMENT

We have found no definite evidence for either a stimulating or an inhibiting humoral factor directly governing hepatic regeneration in several groups of parabiotic rats. Our findings therefore contrast with those of several earlier workers. There are a number of factors which may bear on the differences in our observations. The type of controls utilized is an important consideration. It is possible that parabiotic union itself may have an effect on an organ or parameter under investigation and that parabiotic animals may respond to a procedure differ-

ently from normal animals. For example, a difference in response between parabiotic and single rats is illustrated in Text-figure 2. Here it is shown that sham operated paired and triplet rats exhibited greater hepatic DNA synthesis than single controls. In addition, it was observed in paired rats whose partners had undergone a sham operation that there was significantly increased hepatic mitotic activity 48 and 72 hours after operation but insignificantly greater hepatic DNA synthesis.

Since a stimulating or inhibitory response may follow a sham operation, the reaction to partial removal of an organ must be measured against sham operated controls as well as other control groups, most importantly, nonoperated parabiotic subjects. In the studies previously cited this was not done.[1-3]

An explanation for what may have been a response of hepatic DNA synthesis and mitosis in a parabiotic rat whose partner had undergone a sham operation is not known. One possibility is the transmission between animals of a nonspecific protein material.[11] This is unlikely, however, because it was not noted in triplet parabiotic rats. Sham operation must be investigated further before any conclusion can be reached concerning its effect on cellular proliferation and on growth. In a study of parabiotic triplets not reported here in detail, a small (10 per cent) partial hepatectomy was done in the two lateral members. The findings were the same as with sham operation alone.

Other factors which may be of importance in interpretation are biologic variation, the existence of intraperitoneal or intrahepatic inflammation, or modifications of growth. There are normal variations in the indexes of metabolism and especially of growth. Thus, if two very small groups of animals are used, statistically significant differences may be found on occasion which are not related to the experimental procedure. This is particularly true in our experience in relation to the number of cells in mitosis at any one time in a rat liver. As was pointed out in an earlier publication,[4] small numbers of rats have been used in many studies of hepatic regeneration and in those involving the infusion of plasma or serum. Therefore, different conclusions may have been reached because of biologic variation.

Unpublished studies which we have made have shown that intraperitoneal or intrahepatic inflammation may result in stimulation, or alternating stimulation and depression of hepatic mitosis. Maximal hepatic DNA synthesis and mitotic activity in single rats following partial hepatectomy is known to occur approximately 24 hours after operation.[12] The transmitted response reported in some parabiotic rats, but which has not been found until 48 hours or more after operation, may have been due to increasing inflammation (or related phenomena) spreading into the connected peritoneal cavities.

Finally, consideration must be given to the effect of an operative procedure alone on the continuous process of growth and replacement of cells. Rats grow throughout their lives; a probable reflection of this, together with the factor of cell replacement, is the evidence of constant hepatic parenchymal DNA synthesis and mitosis.[13] These processes are continuous, but they wax and wane in intensity.[13] A wide variety of experimental procedures, especially those of stressful nature, may have a transient effect, especially on the process of mitosis, without leading to the continued growth or regeneration of an organ.[14] Manifestations which affect hepatic DNA synthesis or mitosis temporarily may not represent the same mechanisms that initiate and control hepatic growth and regeneration.

Summary

Regeneration of the liver was investigated in single and parabiotic paired and "triplet" rats following sham operation and partial hepatectomy. Quantitation of mitosis and DNA synthesis was determined with the aid of H^3-thymidine and use of autoradiographs.

Partial hepatectomy in one member of a parabiotic pair and in two members of parabiotic triplets failed to elicit a statistically significant response in hepatic DNA synthesis or mitosis in a nonoperated partner.

Hepatic DNA synthesis was greater in nonoperated parabiotic triplet rats than in nonoperated single rats, although differences were not of statistical significance.

After sham operation in parabiotic paired and triplet rats, greater hepatic DNA synthesis and mitotic activity was found than in single sham operated rats.

After a sham operation in a member of parabiotic paired rats, greater hepatic DNA synthesis and mitotic activity was found in the nonoperated partners.

Partial hepatectomy in parabiotic paired and triplet rats resulted in a regenerative response that was similar to that observed in partially hepatectomized single rats. Variations in the degree of response were found among different groups of parabiotic and single rats.

The present experiments do not support the conclusion that the blood in parabiotic rats carries stimulating or inhibitory "humoral factors" directly governing hepatic regeneration. This does not exclude the possibility that regeneration and growth may be modified by blood flow or by changes in the composition of the blood.

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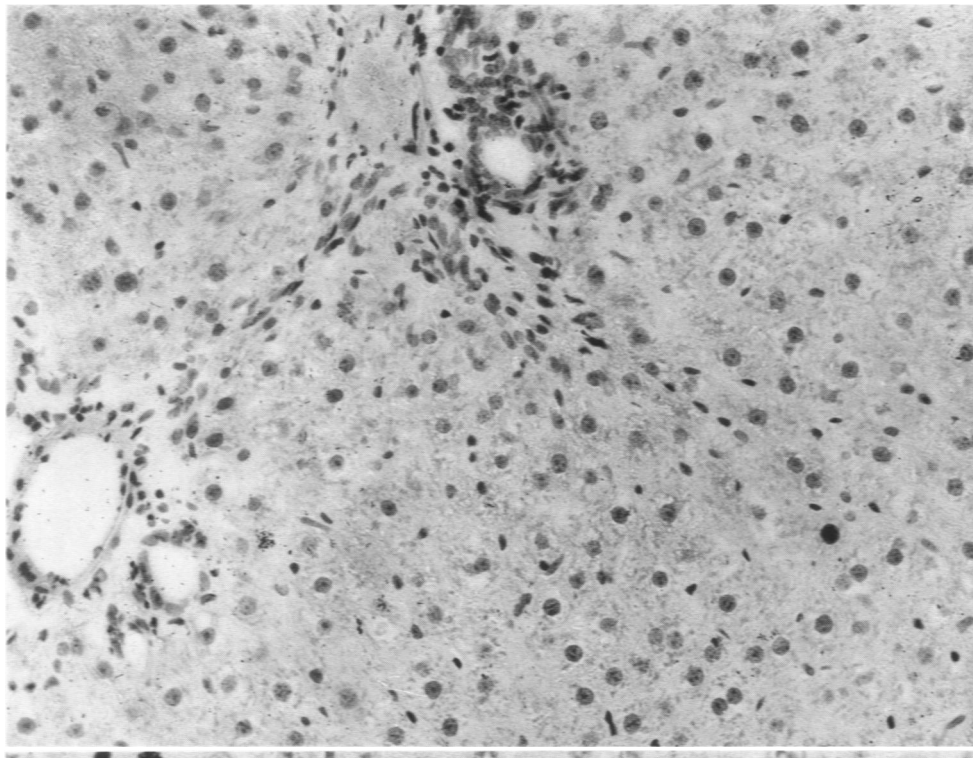
LEGENDS FOR FIGURES

All photomicrographs are autoradiographs of sections stained with hematoxylin.

FIG. 1. Liver of a normal, nonoperated, single rat that received 1 μc. of H^3-thymidine per gm. of body weight intraperitoneally 4 hours before sacrifice. There is one H^3-thymidine labeled hepatic cell nucleus. × 380.

FIG. 2. Liver of a single rat that underwent approximately 70 per cent partial hepatectomy and received 1 μc. of H^3-thymidine per gm. of body weight intraperitoneally 48 hours later, 4 hours before sacrifice. There are approximately 25 labeled hepatic nuclei, and several mitotic figures in hepatic cells. × 380.



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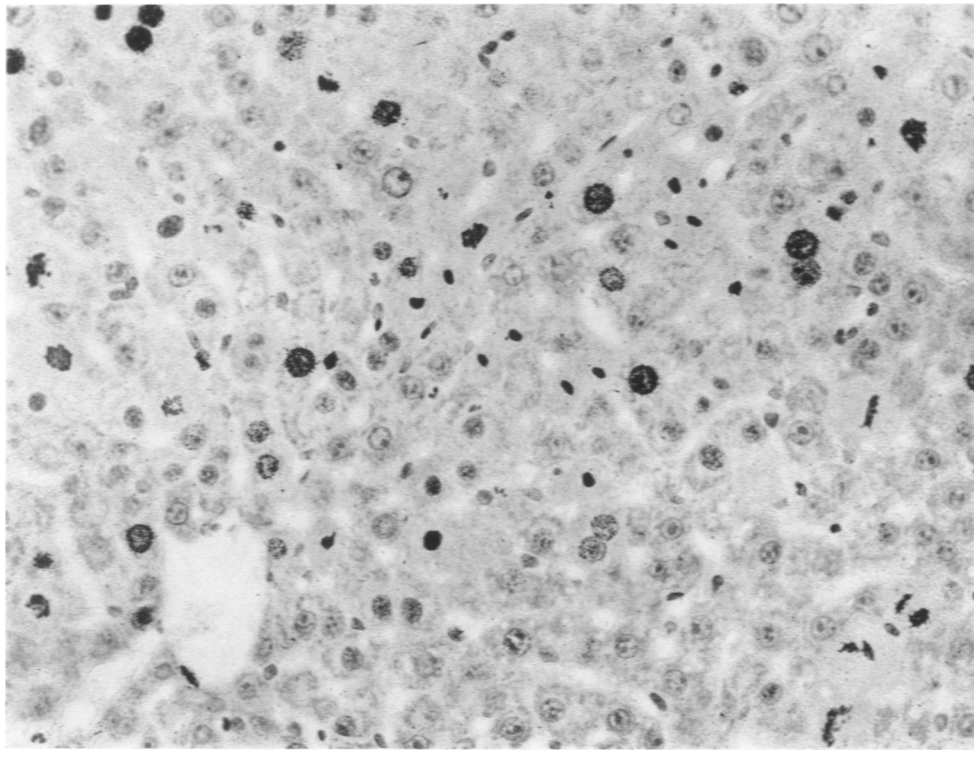

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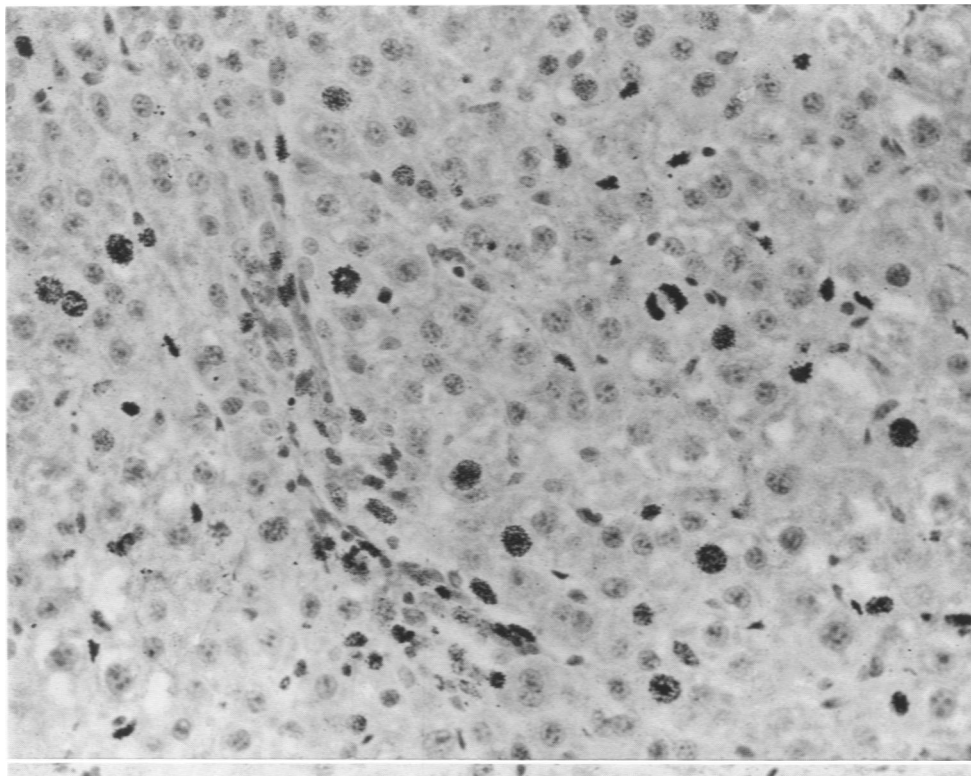
FIG. 3. Sprague-Dawley triplet parabiotic rats at the time of sacrifice. They are healthy, with abundant growth of hair along the lines of incision.

FIG. 4. Liver of a triplet parabiotic rat that underwent approximately 70 per cent partial hepatectomy and received 1 μc. of H^3-thymidine per gm. of body weight intraperitoneally 48 hours later, 4 hours before sacrifice. There are approximately 25 labeled hepatic cell nuclei. comparable to the number in the liver in Figure 2. × 380.

FIG. 5. Liver of a center, nonoperated triplet parabiotic rat whose two partners underwent approximately 60 per cent and 70 per cent partial hepatectomy. All 3 animals received 1 μc. of H^3-thymidine per gm. of body weight intraperitoneally 48 hours later, 4 hours before sacrifice. There are approximately 5 labeled hepatic nuclei and occasional mitotic figures in hepatic cells. × 380.



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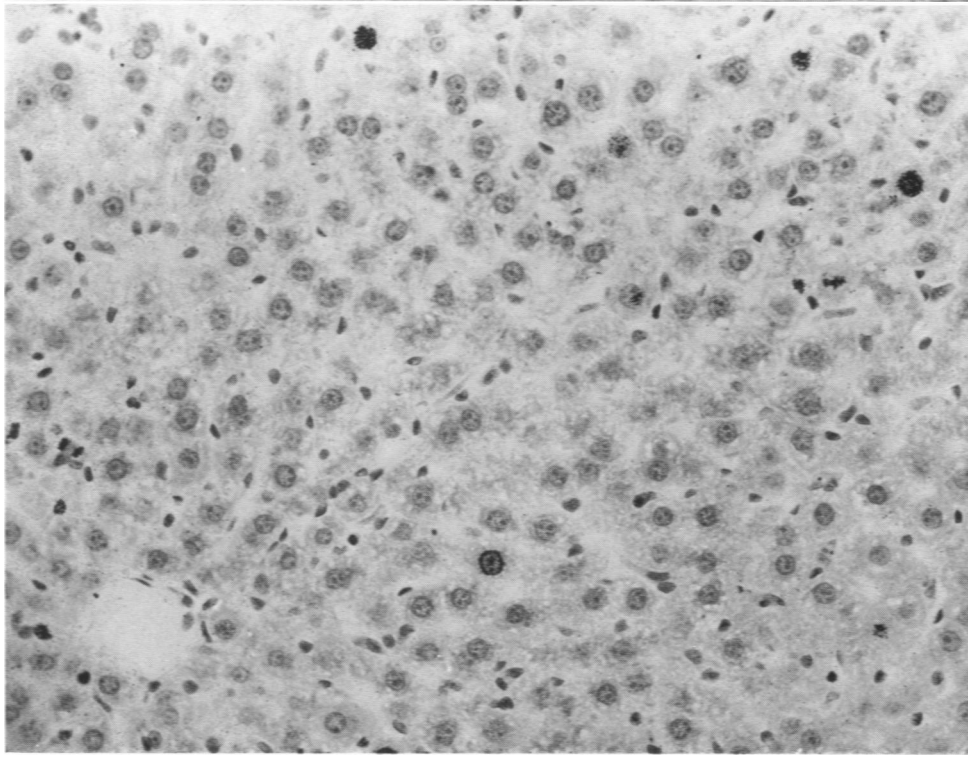

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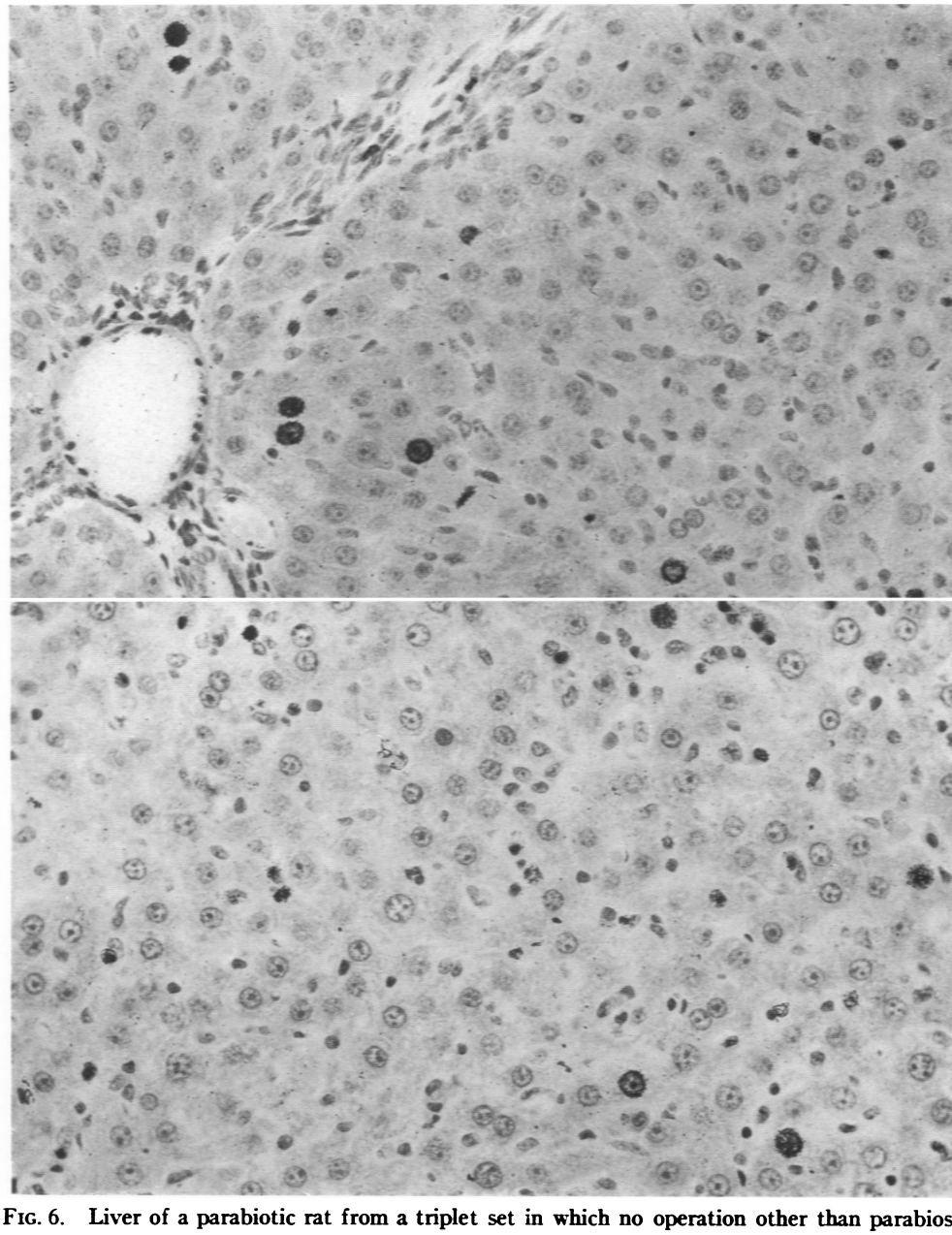

FIG. 6. Liver of a parabiotic rat from a triplet set in which no operation other than parabiosis was performed. All animals received 1 μc. of H^3-thymidine per gm. of body weight 4 hours before sacrifice. There are approximately 7 labeled hepatic nuclei, comparable to the number shown in the liver in Figure 5. This is the greatest amount of H^3-thymidine uptake noted in nonoperated parabiotic rats, and illustrates the considerable variation in DNA synthesis and mitosis encountered in parabiotic animals. × 380.

FIG. 7. Liver of a center, nonoperated triplet parabiotic rat whose two partners underwent sham operation. All 3 animals received 1 μc. of H^3-thymidine per gm. of body weight intraperitoneally 48 hours later, 4 hours before sacrifice. There are approximately 6 labeled hepatic nuclei, comparable to the number in the livers shown in Figures 5 and 6. × 380.