Regeneration of the Liver in Parabiotic Rats*††

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In the adult animal, as is well known, the various tissues of the body exhibit mitotic rates which are quite characteristic, and which seem to be geared to the needs of the organism in providing for the replacement of worn-out cells. Thus, the rate at which cells normally divide is more nearly related to their life expectancy than to their actual growth potential. The latter is, in most instances, far greater than is ever manifested under ordinary circumstances. Relatively little is known about the fundamental mechanisms which are involved in growth regulation of this kind, but they deserve careful study, since lack of such control is one of the chief attributes of cancer.

In the present investigation, an initial attempt has been made to explore the operation of these mechanisms in rat liver. In the normal adult rat the incidence of mitosis is very low; at a given instant not more than one dividing nucleus may be found among 10–20,000—a rate of 0.005–0.01 per cent (5). Following extirpation of approximately one-third of the liver, however, the rate rises to 0.05 per cent.† If two-thirds of the liver are removed, the rate goes up to 2 per cent or more (3) —a 200- to 400-fold increase over the normal resting value. As the number of hepatic cells approaches its original limit, the mitotic rate falls to normal.

We have attempted to localize the mechanism by which this balanced response to physiological needs is maintained. An attempt has been made to ascertain whether factors circulating in the bloodstream during regeneration could serve to initiate the proliferative response to partial hepatectomy, or whether the stimulus is purely local.

In order to detect the presence of blood-borne factors, a series of parabiotic rats was prepared. When the animals had reached a suitable age, and the anastomosis was well healed, one member of each pair was partially hepatectomized. After an appropriate interval, the animals were sacrificed, and the intact liver of the nonhepatectomized partner was examined for alterations known to occur characteristically during hepatic regeneration. In several instances parabiotic triplets were also prepared. In these, partial hepatectomies were carried out on the two end partners, leaving the middle one intact for subsequent examination.

METHODS

The rats used in these experiments were all derived from Wistar stock, bred in our own laboratory for the past 8 years. Males and females were used, but both partners of any one pair were of the same sex. In the first eight pairs the animals were matched as to age and weight but were not littermates; in all subsequent instances they were littermates.

All operative procedures were carried out under ether anesthesia and with the usual aseptic precautions. The fur was removed by shaving or, more frequently, by means of a depilatory (19).

Parabiotic pairs were prepared by either the technic of Bunster and Meyer (5) or by a modification of the open celomic method. By the former procedure, although the peritoneal cavities of both rats were incised, they were closed in such a manner that the abdominal walls were united without celioanastomosis. This latter technic was modified by an additional step; the anterior surfaces of the medial two-thirds of both spleens were lightly scarified with a scalpel and then fixed in apposition by a fine continuous silk suture run through the superior and inferior edges. This procedure was intended to increase the area of healing surfaces between the two rats and so enhance the volume of cross circulation.

Parabiotic triplets were united by the open
celomic method, but the spleens were not joined. The entire operation was carried out in one stage.

In the earlier experiments, partial hepatectomies were performed by the usual techinic, with excision of the median and left lateral lobes (8, 2). Later, to augment the regenerative stimulus by the production of an even greater liver deficiency, part of the right lateral lobe was also removed. After the main lobes had been excised in the routine manner, a heavy (No. 2) silk thread was looped about the upper third of the right lateral lobe, including the distal portions of its two component lappets, tightened, and tied securely. Although by this procedure the ligature tore its way through the middle of the lobe, very little loss of blood resulted, and the distal parts of the lobe could readily be excised.

It was originally demonstrated by Brues et al. (9) that the liver remaining after extirpation of the two main lobes comprised 31.6 ± 1.5 per cent of the total. Good agreement with this result has been obtained in our hands (4). Hence, the weight of the total liver was calculated on the assumption that the median and left lateral lobes constituted 68.4 per cent of its original mass. In those animals in which part of the right lateral lobe was also removed, the main lobes and the additional lobes were weighed separately, and the total original liver mass estimated as usual from the weight of the former. The total percentage excised was then determined on the basis of the combined weights of all the extirpated lobes. By this means the total amount of liver excised was found to comprise from 75 to 88 per cent of that originally present.

The animals were sacrificed under ether anesthesia by exsanguination while the liver was rapidly excised, at intervals of 24, 48, or 72 hours after completion of the partial hepatectomy.

The liver removed at the time of partial hepatectomy served as the control for the intact liver of the nonhepatectomized partner, obtained 1–3 days later at autopsy.

As soon as possible after removal of the liver (either at operation or autopsy), small pieces were fixed in Bouin's fluid for histological study, and other samples were weighed and set aside for determination of total nitrogen, pentosenucleic acid (PNA), desoxypentosenucleic acid (DNA), alkaline phosphatase, and water content.

The rats were denied food for 20–24 hours prior to partial hepatectomy or autopsy, in order to reduce the hepatic glycogen content and thus facilitate the enumeration of mitotic figures in the tissue sections, and also to provide a more uniform condition of the tissue for chemical analysis.

Following fixation, the tissues were imbedded and sectioned at 6 μ. The sections were examined at a magnification of 660X under a microscope fitted with an ocular field-stop which delineated a square 0.17 × 0.17 mm. in the object plane. For each section studied, the average number of hepatic cell nuclei per field was estimated from counts performed on ten such fields. Successive fields were searched for mitotic figures either until 30 mitoses had been found or until a total area equivalent to at least 100,000 nuclei had been examined. Only fields containing liver cells and no sizable ducts or vessels were chosen, so that the results would be uniform. Since it has been shown that the distribution of mitoses is random throughout the liver lobule (3), no error should arise from an arbitrary selection of fields. Only very obvious mitoses were counted—those in late prophase, metaphase, anaphase, or early telophase, and with a large complement of chromosomes present—to preclude enumeration of part of the same figure in an adjacent section. As an added precaution against this possibility, since nuclei undergoing mitosis may be several times as large as the thickness of the section, only every second section was studied. The total number of nuclei was estimated from the average number of nuclei per field and the total number of fields examined. The percentage of nuclei undergoing mitosis at a given instant was calculated from this value and from the total number of mitoses observed. For determination of alkaline phosphatase, the liver samples, which had been stored in glass-stoppered flasks containing 2 drops of chloroform at −10° C., were thawed, finely minced, then ground with washed sand and allowed to stand for 48 hours at 4° C. in 20 volumes of distilled water containing several drops of chloroform (7). After being strained through washed gauze, aliquots of the extract were incubated with M/20 disodium monophenyl phosphate in M/15 glycine buffer and the liberated phenol measured colorimetrically (12). Activity was expressed as milligrams of phenol liberated/gm fresh tissue/hour at pH 9.3 and 37° C.

For determination of nucleic acids, samples of liver which were not processed immediately were either frozen in small chunks by immersion in isopentane cooled in liquid nitrogen to −190° C. and then stored under isopentane at −40° C. to be homogenized later, or they were homogenized immediately in 5 volumes of distilled water and stored at −40° C. as homogenates. The procedure was a combination of the method of Schmidt and Thannhauser (21) with that of Schneider (22). The former method was followed in separating pentosenucleic acid (PNA) from desoxypentosenucleic acid (DNA), by precipitation of the DNA after
alkaline hydrolysis. The PNA, which remained in the supernatant, was determined as pentose by the orcinol reaction (16). The DNA was extracted from the precipitate by treatment with hot trichloroacetic acid according to the method of Schneider (22) and was measured as desoxypentose by the diphenylamine reaction (6). All analyses were performed by the micro-Kjeldahl procedure (19).

The percentage of water present was determined from a known weight of finely chopped fresh liver, which was dried to constant weight at 78°C. and a pressure of 0.05 mm. Hg. All analyses have been expressed in terms of fresh liver weight.

RESULTS

Parabiotic pairs without celioanastomosis.—The first group of parabiotic rats consisted of seven pairs of animals that were not littermates. They were united at 2-3 months of age, without celioanastomosis, by the technic of Bunster and Meyer. Seven months later approximately 68 per cent of the liver was removed from the partner on the right of each pair. Two pairs were sacrificed at 48 hours, and the rest at 72 hours after the partial hepatectomy.

At autopsy, the first three pairs of rats (Nos. 6, 7, and 8 in Table 1) were found to be loosely joined, the union involving little more than the skin, which was not well vascularized. These three pairs have been omitted from the final analysis of the results.

The autopsy findings in the remaining four pairs revealed a more effective type of union, with closer apposition of scapulas and junction of part of the abdominal musculature, except in pair No. 15. In this pair no grossly visible connection was observed between muscle layers, and a relatively low mitosis count was obtained in the liver of the nonhepatectomized partner (Table 1).

In each of these four pairs, the percentage of dividing nuclei was higher in the liver of the nonhepatectomized partner than in the control, with a mean of 3.6 times the control level, in spite of the abnormally high value found in control No. 10 for which no obvious explanation could be found.

Parabiotic pairs with celioanastomosis and splenic juncture.—This group consisted of five pairs of rats, all but the first of which were littersmates. Parabiosis was produced at between 21/2 and 3 months of age and involved open celomic anastomosis and junction of spleens. After 11/2-21/2 months, 68 per cent of the liver was removed from the right partner of each pair. Two pairs were sacrificed at 24 hours, one at 48 hours, and two at 72 hours after the hepatectomy.

At autopsy all the pairs appeared to be well joined, with skin and muscle layers firmly united. The spleens were in good apposition, and the surfaces were adherent, although the passage of blood vessels through the juncture could not be positively demonstrated microscopically.

In the two pairs of rats sacrificed 24 hours after hepatectomy, the intact livers of the nonhepatectomized partners exhibited no increase in mitotic rate as compared to the controls (Table 1). This finding was anticipated and is in keeping with the observation that mitosis is not increased in regenerating liver during the first day after partial hepatectomy (3).

In the remaining three pairs, the intact livers of the nonhepatectomized partners all exhibited an increase in mitosis, the mean rate being 11 times that of the control animals (Table 1).

Parabiotic pairs with celioanastomosis and splenic juncture given colchicine.—In the third group of parabiotic pairs, an unsuccessful attempt was made to augment the effect obtained in the first two groups. A larger fraction of the liver was excised in the hope of producing a stronger stimulus, and colchicine was administered 8-18 hours before sacrifice in order to produce an accumulation of mitoses and to facilitate counting.

Table 1

<table>
<thead>
<tr>
<th>Age at hepatectomy (months)</th>
<th>Age at sacrifice (months)</th>
<th>Per cent mitosis in control</th>
<th>Per cent mitosis in intact partner</th>
<th>Ratio-partner/control</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6</td>
<td>3</td>
<td>10</td>
<td>0.0018</td>
<td>0.0059</td>
</tr>
<tr>
<td>P7</td>
<td>3</td>
<td>10</td>
<td>0.0003</td>
<td>0.0058</td>
</tr>
<tr>
<td>P8</td>
<td>3</td>
<td>10</td>
<td>0.0027</td>
<td>0.0058</td>
</tr>
<tr>
<td>P10</td>
<td>3</td>
<td>10</td>
<td>0.0059</td>
<td>0.0058</td>
</tr>
<tr>
<td>P9</td>
<td>3</td>
<td>10</td>
<td>0.0042</td>
<td>0.0058</td>
</tr>
<tr>
<td>P14</td>
<td>2</td>
<td>9</td>
<td>0.0051</td>
<td>0.0143</td>
</tr>
<tr>
<td>P15</td>
<td>2</td>
<td>9</td>
<td>0.0019</td>
<td>0.0072</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td>0.0066</td>
<td>0.0221</td>
</tr>
</tbody>
</table>

* P6, P7, and P8 not included in this mean value (see text).
† P8 and P14 not included in this mean value (see text).
There were five pairs of rats in this group, all littermates, united at between 3 weeks and 1 month of age by celioanastomosis and splenic juncture. Partial hepatectomies were performed 2, 4, or 12 months later, and instead of the usual 68 per cent, approximately 80 per cent of the liver was excised from the right partner of each pair. The use of colchicine in this experiment necessitated the use of separate controls, since it was undesirable to administer the drug twice to the same pair of rats. The controls in this instance were intact separate rats of the same age and sex as the corresponding parabiotic pair, injected with colchicine and subsequently sacrificed at the same time. Three pairs were sacrificed at 48 hours and two pairs at 72 hours after the hepatectomy. The dose of colchicine was 0.02 mg. in the first three and 0.10 mg. in the latter two pairs, as noted in Table 2; both rats in each pair were injected with these amounts of the drug.

In the two pairs receiving the high dosage, both of the hepatectomized partners were found dead at the time the nonhepatectomized partners were sacrificed; however, this dosage appeared to be well tolerated by the latter and by the controls. The manifest toxicity of the drug in liver-deficient animals may have seriously limited the size of the mitotic stimulus transmitted to the intact partner. Nevertheless, in all five instances in this group, mitoses in the livers of the nonhepatectomized partners were more numerous than in their corresponding controls, the average value for the former exceeding the latter by more than fourfold (Table 2). Thus, while no augmentation of the previous effect was achieved, these findings confirm the positive results obtained in the first two groups.

The results of these experiments are shown graphically in Chart 1.

Parabiotic triplets with celioanastomosis.—Another means of increasing the relative amount of liver deficiency was afforded by the use of parabiotic triplets, in which partial hepatectomies could be performed on two rats out of three. Triplets were prepared from littermates united by celioanastomosis at 1-1½ months of age. Three sets survived in good condition and were subjected to partial hepatectomy at 2, 4, and 6 months of age, respectively. From 75 to 80 per cent of the liver was removed from the two end partners of each set, leaving the middle one intact. All three sets were sacrificed 48 hours after the partial hepatectomies.

The results were striking (Table 3). The percentage of nuclei in mitosis was from 44 to 62...
times the mean control value for each individual set, or a mean increase of 50-fold. The results of all the above experiments are summarized graphically in Chart 2.

Sham-hepatectomized and parabiotic controls.—Four normal, separate female rats, 4 months of age, were subjected to sham operations which simulated a partial hepatectomy; laparotomies most part too slight to be reflected by gross alterations in chemical composition. The water content, total nitrogen, alkaline phosphatase, and DNA all remained within normal limits in the livers of the nonhepatectomized partners. The PNA showed a suggestive but not highly significant rise in the experiments with parabiotic twins (0.02 < P < 0.05), but not with the triplets. In the lat-

TABLE 3

<table>
<thead>
<tr>
<th>Age at parabiosis (months)</th>
<th>Hrs. from heparatectomy to sacrifice</th>
<th>Per cent of liver mitosis in controls</th>
<th>Per cent mitosis in intact partner</th>
<th>Ratio partner/controls</th>
</tr>
</thead>
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<tr>
<td>P97 Right</td>
<td>4</td>
<td>48</td>
<td>79.2</td>
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<tr>
<td>Left</td>
<td>4</td>
<td>48</td>
<td>80.1</td>
<td>0.0163</td>
</tr>
<tr>
<td>Middle</td>
<td>4</td>
<td>48</td>
<td>0</td>
<td>0.5123</td>
</tr>
<tr>
<td>P41 Right</td>
<td>6</td>
<td>48</td>
<td>77.8</td>
<td>0.0086</td>
</tr>
<tr>
<td>Left</td>
<td>6</td>
<td>48</td>
<td>77.5</td>
<td>0.0085</td>
</tr>
<tr>
<td>Middle</td>
<td>6</td>
<td>48</td>
<td>0</td>
<td>0.1546</td>
</tr>
<tr>
<td>P58 Right</td>
<td>3</td>
<td>48</td>
<td>79.1</td>
<td>0.0038</td>
</tr>
<tr>
<td>Left</td>
<td>3</td>
<td>48</td>
<td>75.1</td>
<td>0.0009</td>
</tr>
<tr>
<td>Middle</td>
<td>3</td>
<td>48</td>
<td>0</td>
<td>0.1473</td>
</tr>
</tbody>
</table>
| AV.                       |                                     |                                     | 0.0067                           | 0.3588                 | 50

were performed, followed by manipulation of the liver, and excision of a very small biopsy. The animals were sacrificed 48 hours later.

The mean mitotic rate in the livers of these rats was found to be 0.0025 per cent (Table 4), as compared to 0.0057 per cent for the controls in all the parabiotic series except those which received colchicine. Thus, the increase in mitosis found in the nonhepatectomized partners in the above experiments could not be attributed to factors attendant upon the operative procedure per se. The difference between the mean value for these normal, separate controls and that for the parabiotic controls is probably unimportant, since 0.0057 per cent is within the range reported elsewhere for normal adult rats (3).

Although the basic mitotic rate was not altered by the state of parabiosis, approximately 20 per cent of the livers in these animals exhibited under-development or atrophy of the caudate lobes. This anomaly appeared sometimes in either partner, and sometimes in both. Whatever its cause, since long intervals of time elapsed between the parabiotic union and the final stage of the experiment, no interference with the eventual outcome seemed likely.

Results of biochemical studies.—The results of the biochemical studies are shown in Table 5 and will be summarized very briefly, since the changes produced in the intact liver of the nonhepatectomized partner in these experiments were for the...
TABLE 5
PER CENT RESTORATION OF LIVER MASS, AND DNA, PNA, ALKALINE PHOSPHATASE, TOTAL NITROGEN AND WATER CONTENT OF CONTROL, REGENERATING, AND INTACT LIVERS OF PARABIOtic TWINS AND TRIPLETS

<table>
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<tr>
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<tbody>
<tr>
<td>Rate No.</td>
<td>DEX</td>
<td>PENT</td>
<td>ALK</td>
<td>DEX</td>
<td>PENT</td>
<td>ALK</td>
</tr>
<tr>
<td>P28</td>
<td>24</td>
<td>43.2</td>
<td>155</td>
<td>121</td>
<td>102</td>
<td>1,068</td>
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<tr>
<td>P24</td>
<td>24</td>
<td>44.7</td>
<td>155</td>
<td>121</td>
<td>102</td>
<td>1,068</td>
</tr>
<tr>
<td>P8</td>
<td>48</td>
<td>48.6</td>
<td>128</td>
<td>124</td>
<td>238</td>
<td>922</td>
</tr>
<tr>
<td>P10</td>
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<td>55.0</td>
<td>254</td>
<td>254</td>
<td>254</td>
<td>744</td>
</tr>
<tr>
<td>Mean††</td>
<td>50.7</td>
<td>204±80.7</td>
<td>246±10.6</td>
<td>204±80.7</td>
<td>246±10.6</td>
<td>204±80.7</td>
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<tr>
<td>P7</td>
<td>72</td>
<td>53.0</td>
<td>196</td>
<td>150</td>
<td>235</td>
<td>783</td>
</tr>
<tr>
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<td>72</td>
<td>62.2</td>
<td>169</td>
<td>128</td>
<td>221</td>
<td>908</td>
</tr>
<tr>
<td>P9</td>
<td>72</td>
<td>68.2</td>
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<td>128</td>
<td>221</td>
<td>908</td>
</tr>
<tr>
<td>P14</td>
<td>72</td>
<td>68.7</td>
<td>116</td>
<td>224</td>
<td>700</td>
<td>655</td>
</tr>
<tr>
<td>P15</td>
<td>72</td>
<td>66.0</td>
<td>230</td>
<td>104</td>
<td>203</td>
<td>785</td>
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<td>P25</td>
<td>72</td>
<td>61.3</td>
<td>106</td>
<td>170</td>
<td>631</td>
<td>987</td>
</tr>
<tr>
<td>P26</td>
<td>72</td>
<td>53.0</td>
<td>152</td>
<td>235</td>
<td>618</td>
<td>976</td>
</tr>
<tr>
<td>Mean††</td>
<td>63.4</td>
<td>175±15.0</td>
<td>145±17.1</td>
<td>221±8.8</td>
<td>175±15.0</td>
<td>145±17.1</td>
</tr>
</tbody>
</table>

Twins: Buettner and Meyer or Open CeloMico-Splenic Technique; 68 Per Cent Hepatectomy

Triplets: Open CeloMico Technique; 80 Per Cent Hepatectomy

* Calculated according to the method of Brusco, Drury, and Brusco (2).
† Mg/100 gm of fresh tissue.
‡ Standard error of the method = ± 7.5 per cent.
§ Standard error of the method = ± 2.9 per cent.
|| Units = mg of phenol liberated/gm of fresh tissue/hr at pH 9.3 and 37°C.
## Standard error of the method = ± 2.1 per cent.
### Standard error of the method = ± 0.9 per cent.
†† Mean ± standard error of the mean.
ter, the state of parabiosis appears to be attended by more sweeping alterations in nutritional status and other physiological functions than in the former. Hence, in triplets the pattern of the biochemical responses of the liver to the regenerative stimulus may not necessarily be the same as in the twins.

It should be noted that the mitotic rate, which can increase by 400-fold during hepatic regeneration, is at least 50 times more sensitive as an indicator of regenerative activity than any of the biochemical tests employed.

Results obtained in the regenerating livers of partially hepatectomized partners.—The effect of parabiosis on the regenerative process in the partially hepatectomized partners could best be evaluated in the animals that were subjected to 68 per cent hepatectomies, since the response in separate rats has been widely studied under these circumstances and is predictable. Restoration of liver mass (Table 5) was found to proceed at the same rate as that obtained in previous experiments in this laboratory in which normal separate rats were studied (4). Mitotic figures were not counted, but they were present in sufficient numbers to indicate that active proliferation was in progress. The rate of restoration of hepatic nuclei (3, 4) determined in one liver that had been regenerating for 72 hours (Rat 25, Table 1) was found to be at the expected level for separate rats (43 per cent). The biochemical alterations exhibited by these regenerating livers (Table 5) were likewise consistent with those reported to occur in hepatic regeneration in normal separate rats (2, 17, 18).

In the animals subjected to 80 per cent hepatectomies, the progress of restoration was more difficult to evaluate, because of lack of a suitable reference standard. The regenerating remnant increased in size and became excessively fatty, assuming a pale cream color. On microscopic examination, mitotic figures were very numerous, but whether the triplets differed significantly from the twins in this respect could not be determined because of the variability in the amount of liver removed by this technic.

In general, the results implied that the proliferative response in the livers of the intact partners was not of sufficient magnitude to inhibit appreciably the regenerative response in the hepatectomized partners.

DISCUSSION

The above experiments have demonstrated that excision of part of the liver from one parabiotic partner was followed in every instance by a proliferative response, not only in the liver of the partially dehepatized rat but also, though to a lesser degree, in the intact liver of the other partner. These findings provide evidence in favor of the existence of a blood-borne cog in the mechanism for the initiation of liver mitosis.

Although the results were consistently positive, the nonhepatectomized partners in all groups exhibited considerable variation in the degree of this mitotic response. Similar variability has been noted in the regenerating livers of normal separate rats in which it was observed that the percentage of cells undergoing mitosis fluctuated widely from hour to hour and at different times in different individuals (3). Differences in the efficiency of the cross-circulation between partners may have also contributed to the variability of our results. Even when the same technic of anastomosis has been employed, the rate of blood exchange between partners varies somewhat in different pairs of rats (1, 25). For an agent to serve effectively in carrying a stimulus from one partner to another via the blood stream, the rate of transmission from donor to recipient must exceed the rate of elimination in the recipient partner by an amount sufficient for an above-threshold concentration of the active substance to obtain in the latter (11). In our experiments with parabiotic twins, where the stimulus was not very great, small differences in the volume of blood exchanged may have exerted an appreciable effect upon the degree of response exhibited by the nonhepatectomized (i.e., recipient) partner.

With parabiotic pairs, the results, although definite and clear-cut, were not striking. The mean mitotic rate for the livers of nonhepatectomized partners in all groups of twins (0.03 per cent) exceeded that of the controls (0.005 per cent) by approximately sixfold. If 68—80 per cent of the liver is removed from one rat of a parabiotic pair, the total liver mass removed from both is only 34—40 per cent. Thus, the rise in mitotic rate to 0.03 per cent in the nonhepatectomized twins is compatible with the finding of Hempelmann, previously mentioned, when only one-third of the liver was removed from a single rat, the mitosis rate approached 0.05 per cent—as opposed to 2 per cent when two-thirds were removed.

A considerable augmentation of the positive effect obtained in the experiments with parabiotic twins occurred with triplets. In the latter, since 80 per cent hepatectomies were performed on two rats out of three, the total liver mass removed from the trio was approximately 55 per cent—an appreciable increase over the 34—40 per cent

1 L. H. Hempelmann, personal communication.
removed from the twins. In addition to the larger stimulus provided by the greater liver deficiency, a more effective cross-circulation should occur in triplets, since the anastomosis in the middle partner is twice as extensive as in twins. It appears from these data, as well as from those of Hempelmann cited above, that a large stimulus is necessary in order to obtain a substantial response.

To insure against unpredictable alterations in the mitotic rate that might arise as an accompaniment of the parabiotic state, one partner of each pair was used as the control for the other. Thus, any effect resulting from parabiosis itself would be reflected in the control. The atrophy of the caudate lobes, exhibited by many rats in our series, had no detectable effects upon the mitotic rate, since the control rats in which it occurred exhibited mitotic counts well within normal limits. Hence, the presence of this anomaly could not be accountable for any significant experimental error.

In the present experiments, the parabiotic technic has been employed to explore the role of the blood stream in transmitting the mitotic stimulus which initiates hepatic regeneration under physiological conditions. Significant studies on the relation of circulation to hepatic regeneration have been previously reported by Mann and his co-workers. In an ingenious series of experiments involving Eck fistulas, partial and reverse Eck fistulas, and partial ligation of the portal vein, they demonstrated that the amount of regeneration was related directly to the amount of portal blood flow and did not depend upon the physiological needs of the organism (14, 9, 24, 10, 15).

The marked distention of the sinusoids in the liver remnant, resulting from the necessity of accommodating the normal volume of portal blood, was believed to induce a coincident hyper trophy of the hepatic cells, with an accompanying increase in mitosis. A second possible interpretation of these findings, however, is that rather than purely mechanical distention of the sinusoids by an added flow of portal blood, there are chemical entities in the blood which, when present in sufficient amounts, may cause a proliferative response in the liver. Thus, it is conceivable that either an elevated concentration of such substances or an increased flow of blood bearing the same concentration would be equally effective. Our results tend to support this second interpretation, since we do not believe that the volume of blood circulating through the intact liver of the nonhepatectomized partner has been altered in our experiments. In only one instance was there any evidence of an adhesion, and that was between the liver and spleen of one of the rats in a colchicine-treated pair. In all other cases, and particularly in the animals joined by the Bunster and Meyer technic without celioanastomosis, no adhesions were detected between the organs drained by the portal system and the body wall. Such adhesions would have to be quite extensive, and present in both partners, to produce an effective increase in blood flow through the liver of one partner as a result of a hepatectomy in the other.

SUMMARY AND CONCLUSIONS

Partial hepatectomies have been performed on one partner of each of fourteen parabiotic pairs, and on two partners of each of three sets of parabiotic triplets.

At intervals of 48 or 72 hours later, the intact livers of the nonhepatectomized partners all exhibited a higher mitosis rate than the control livers removed at the time of hepatectomy. In parabiotic twins the mean value was 6 times, and in triplets it was 50 times, that of the controls.

Two of the fourteen pairs were sacrificed at 24 hours, and, in these, no significant alteration occurred in the livers of the nonhepatectomized partners.

Biochemical determinations of alkaline phosphatase, total nitrogen, DNA, and water content failed to demonstrate significant differences. PNA values were increased slightly above the control levels in the nonhepatectomized partners of the twins but not in the triplets. All these entities are far less sensitive indices of proliferative activity than the mitotic count.

The evidence suggests that in regenerating rat liver, mitosis is initiated by alterations in the chemical composition of the blood.

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