Regeneration of Liver Tissue Following Partial Hepatectomy in Parabiotic Rats. (18594)

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Liver regeneration in single albino rats has been observed as follows by Higgins(1). Rats were partially hepatectomized and killed by exsanguination at intervals up to 30 days postoperatively. The livers were weighed at autopsy and the percentage of regeneration calculated on the basis of liver to body weight ratios in a series of control rats. Franseen(2) employed a standardized procedure, removing 66.83% ± 3.54 of the liver by excising the median and left lateral lobes and leaving the posterior lobe. We confirmed Franseen's observations by performing such partial hepatectomies (lobectomy) on 5 rats, immediately killing them, weighing the remaining liver, and calculating the percentage of the liver removed. This turned out to be 67% ± 5, and the identical operation was then utilized in our experiments. We consider this to be a better measure of the percentage of liver removed than is given by comparing the weight of removed liver with the average of total liver weight of a control series of rats of comparable body weight.

The purpose of the present work was to observe liver regeneration in parabiotic rats as compared with a group of single-animal controls. Lobectomies were performed on 1 animal in each of 8 parabiotic pairs, and on 16 single control rats. Regeneration was based upon wet weights of liver estimated preoperatively, or measured at time of hepatectomy and at autopsy. Mitotic figures of liver tissue at time of lobectomy and at sacrifice were also studied.

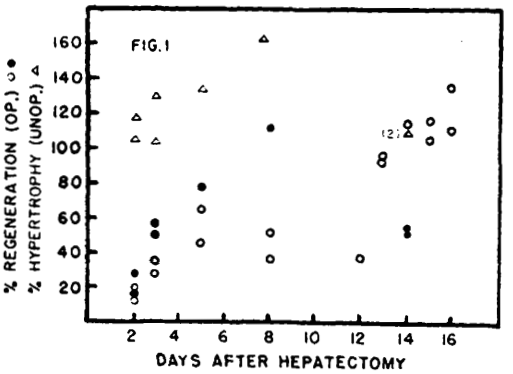
Method. The range of body weight used in our rat group was from 125-225 g. The parabioses were performed on Anheuser-Busch rats, paired as closely as possible by weight and color. Successful parabioses were obtained with pairs of males and pairs of females; no attempts were made to pair animals of opposite sex. A median incision was made from the xiphoid process 3-4 cm caudad. Ligaments binding the left lateral lobe to the posterior lobe and median lobe to the diaphragm were severed. Branches of the hepatic artery and portal vein were ligated with No. 2 chromatized catgut, and the left lateral and median lobes removed *in toto*, with only slight loss of blood. The animals were then sutured in two layers. This operation was performed on one animal in each parabiotic pair and on the single animal control group. Animals were fed chow biscuits and water during the experiment, with 20% glucose added to the drinking water of the hepatectomized animals for 1 postoperative day. Lobectomies were done 5-6 days after the parabiosis operation, since cross-circulation is established by the fourth postoperative day(3). Nembutal, 35 mg/kg intraperitoneally, was used in the parabiosis operations, and ether in the lobectomies. About 200,000 units of penicillin subcutaneously or intramuscularly was administered daily for 2-3 days after every operation. At various intervals up to 14 days following lobectomy the parabiotics were killed by exsanguination, separated and each parabion weighed. The liver weight of each animal in the union was recorded and sections made. The sections were placed in Bouin solution within 6 minutes after removal from the body.

Results. The percentages of regeneration and of hypertrophy of the operated and unoperated livers, respectively, are plotted in Fig. 1. The percentage of regeneration of liver tissue in the lobectomized animals is that based on the fragment of liver removed,

1. Higgins, G. M., Anderson, R. M., *Arch. Path.*, 1931, v12, 186.

2. Franseen, A. M., Brues, A. M., Richard, R. L., *Endocrinology*, 1938, v23, 292.

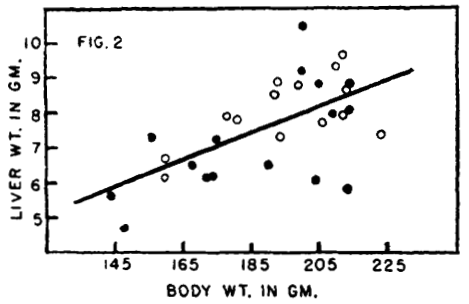
3. Moore, R. A., Hellman, L. M., Jacobius, H., *Arch. Path.*, 1942, v34, 196.



Open and closed circles represent the percentage of regeneration occurring in the single and parabiotic lobectomized animals, respectively. Regeneration is based upon the fraction of the liver removed as described in the following formula:

$$\frac{\text{\% regeneration} = \text{Wt liver at autopsy} - \tfrac{1}{2}\text{ amount removed}}{\text{Amount removed}} \times 100$$

where $\frac{1}{2}$ amount removed = amount remaining after lobectomy. Triangles represent the hypertrophy over normal occurring in the unoperated animal of the parabiotic union. The normal liver weight is based upon calculations made from Fig. 2.



Closed circles represent liver weights obtained by killing animals by exsanguination, followed by measuring the wet weights of their livers. Open circles represent liver weights determined by weighing the liver removed at time of lobectomy on the single animal control group, liver weight being 3/2 times the amount removed.

as explained in the legend. Thus, there has been 50% regeneration when the remaining liver tissue has increased so that half of the removed tissue has been replaced. The percentage of regeneration in our single animals follows fairly closely that obtained by Higgins. Regeneration in the earlier stage seems to occur at a faster rate in the parabiotic than in the control group, but since there are 2 exceptions at the 14 day period, the small size of the experimental group limits any generalization.

However, the liver weights found at autopsy in the unoperated parabions merit attention. Of the 8 parabiotic pairs used, the livers of the unoperated rats were without exception larger than normal. In fact, at the 8 day interval, one liver weight is recorded as being over 160% of the normal liver weight for that size rat. The curve in Fig. 2 was used to determine normal liver weights in the unoperated rats, since there was no other way of knowing the weights of these livers at time of union.

A microscopic study of the unoperated livers at time of sacrifice revealed no congestion, but hypertrophy of the parenchymal cells. Mitotic figures were counted in 10,000 or more liver cells taken at random in each of the unoperated livers and are tabulated as the number of mitoses per 100,000 liver cells.

	Days after hepatectomy				
	2	3	5	8	14
No. of mitoses per 100000 liver cells	150 200	220 60	64	350	6 4

Our counts were higher than those of Christensen(4) who obtained mitotic figure counts of 4, 30, and 33 per 100,000 liver cells on 3 parabions 3 days after hepatectomizing one in each pair. He does not report on weight changes. We observed no mitoses in 10,000 liver cells counted in each of 3 normal liver control slides.

In Fig. 2 a regression line of normal liver weights plotted against body weights in single unoperated animals was determined for our colony of animals. The plotting of points for this line was derived from two sources. Sixteen of the liver weights plotted were determined by weighing the animals, killing them by exsanguination, and measuring the wet weight of the livers. The determination of the other 14 liver weights was based upon the amount of liver removed from our single animal control group. Since 2/3 of the liver was removed at lobectomy, the total liver

4. Christensen, B. G., Jacobsen, E., *Acta Med. Scand.*, 1949, Supplementum 234, 103.

weight is 3/2 the weight of the liver removed. Fig. 2 shows no discrepancy between these 2 sets of points, which further substantiates Franseen's work. From the points plotted in Fig. 2, the slope of this line was derived from the formula,

$$M = \frac{\Sigma xy - n\bar{x}\bar{y}}{\Sigma x^2 - n\bar{x}^2},$$

where the regression line passes through the mean point $(\bar{x}, \bar{y})$. The slope was found to be 0.0364 and the mean point (190.6, 7.613). The equation, therefore, is as follows: $y = 0.0364\ (x - 190.6) + 7.613$.

All but one parabiotic pair had lost various amounts of body weight by the time of autopsy. All of the rats were growing and the difficulty arose as to which body weight was to be used in Fig. 2 to determine what normal liver weight would have been at time of autopsy. We could not apply Jackson's data (5) as to age and duration of inanition to our experiments. The largest weight the rat had ever attained was therefore used to obtain predicted normal liver weight. If the smaller body weights were used, the ratio of liver weight at autopsy to calculated normal liver weight would be even greater than recorded.

To test the validity of the above comparison the ratio of liver to body weight both in normal animals and in unoperated parabiotics was determined. The mean ratio for the normal group was .040 ± .001* and for the experimental group .049 ± .003. The normal group contained 30 and the experimental group 8 animals. Applying the t-test for the comparison of two groups of different sizes, the probability that the difference occurred by chance is less than 2%.

Immediate postoperative mortality was very low in the parabiotic operations. However, many of the rats once in parabiosis died within the 5 days allowed between the first and second operations. These deaths were due to infections and incompatability. The overall mortality was more than 50%. Mortality due to lobectomies on both control and parabiotics was less than 1%.

Discussion. The parabiosis technic afforded a method of introducing a normal liver into the circulation of a partially hepatectomized rat. It was thus possible to observe the effect of this normal liver on one that regenerates, and the effect of the regenerating liver on the normal. Two factors must be present for growth to occur, (1) a response to a growth stimulus and, (2) nutritive materials without which growth cannot occur. Partially hepatectomizing a normal rat initiates a stimulus for liver growth. One cannot say whether this stimulus is due to the introduction of a new humoral factor, or to a humoral factor normally present but increased in amount due to failure of normal destruction by the liver. However, this experiment does indicate that the liver growth factor is humoral, and that a normal liver can respond to it just as does an operated liver, since the liver of the unoperated parabion responded to a substance which must have been humoral.

If the supply of nutrient material is a limiting factor in the rate of regeneration of the operated liver, the introduction of a normal liver into the joined circulation should bring about an increase in nutrient supply to the operated animal, with a resultant increase in the rate of its liver regeneration above that in the single hepatectomized animal. On the other hand, the presence of a normal liver might result in increased destruction of the growth-stimulating factor. Since in this experiment we cannot conclude that regeneration occurred faster in the lobectomized parabions than in the lobectomized single animals, both or neither of the above factors may be at work.

Summary. 1. When one rat of a parabiotic pair was partially hepatectomized, the unoperated liver of its partner increased in wet weight and in the number of hepatic cells. The growth stimulus causing this hypertrophy of liver tissue must have been a factor carried in the blood from the operated animal to the unoperated liver, and the latter responded to

5. Jackson, C. M., *J. Exp. Zool.*, 1915, v19, 99.

* Stand. dev. of the mean $= \sqrt{\frac{\Sigma x^2}{n\,(n-1)}}$.

this stimulus just as does an operated liver. 2. No significant difference was observed between the rates of regeneration of livers in lobectomized parabions and in lobectomized single animals.

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Metabolites of Thyroxine.* (18595)

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The recognition of thyroxine as the thyroid hormone(1,2) makes it important to study its products of metabolism. In the present work, the radioactive substances occurring in the body after administration of radio-thyroxine were detected by radioautography of paper chromatograms of tissues and excreta.

Methods. Radioactive L-thyroxine was prepared by biologically labeling its iodine as described earlier(3). This radio-thyroxine was injected subcutaneously into (a) 6 intact C_3H mice, (b) 3 thyroidectomized C_3H mice, and (c) 3 intact rats. Each animal of these experimental series was injected every 2 hours over a 12-hour period and sacrificed 13 hours after the last injection. The individual doses varied from 0.05 to 0.15 μg of thyroxine and contained from 1 to 5 μc of radioactivity. The purity of similar radio-thyroxine was found to average 92%(3). The nature of the 8% contamination was investigated by chromatographing the injection material according to the method described below. The contaminants were found to consist of traces of iodide and small amounts of an unidentified substance, hereafter referred to as compound No. 1. To remove these contaminants as completely as possible, a sample of radio-thyroxine was chromatographed, the radio-thyroxine spot cut out, eluted, and the elutant injected into one of the 3 thyroidectomized C_3H mice. This procedure successfully removed all of the iodide and all but minute traces of compound No. 1. For comparison purposes, 12 rats and 1 mouse were respectively injected with 100 and 20 μc of *radio-iodide* by the subcutaneous route. They were sacrificed 48 hours later. Liver, kidney, muscle and feces of each of the injected animals were homogenized in volumes of saline in the ratio of 10 cc per gram of tissue. Plasma and urine needed no homogenization and were used as such. All samples were first extracted with twice their volume of water-saturated butanol and then reextracted with an equal volume of the same solvent. These 2 butanol extracts were combined and concentrated to a small volume of *vacuo*. Aliquots were chromatographed on Whatman No. 1 filter paper along 2 dimensions and the resulting chromatograms were subsequently radioautographed. The technical details and the method used for the identification of the spots on the radioautographs have been reported elsewhere(2,4). Preliminary chromatographic runs yielded similar qualitative results for the starting materials (whole plasma, kidney homogenates in saline, etc.) and the corresponding butanol extracts. Since butanol extracts were easier to handle, they were adopted for routine work.

Results. The injection of *radio-thyroxine* gave similar results in intact and thyroidectomized animals. Plasma radioactivity was predominantly in the form of thyroxine (Fig. 1), but there were also small amounts of iodide and of an unidentified substance located to

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1. Leblond, C. P. and Gross, J., *J. Clin. Endocrinol.*, 1949, v9, 149.

2. Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J., *Science*, 1950, v111, 605.

3. Gross, J., Leblond, C. P., *J. Biol. Chem.*, 1950, v184, 489.

4. Gross, J. and Leblond, C. P., *Endocrinology*, 1951, in press.