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ON THE REGENERATIVE PROCESS OF THE HEPATIC PARENCHYMAL CELLS IN PARABIOTIC RATS

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In order to investigate on humoral factors concerning regeneration and proliferation of cells, histological, histochemical and micrometrical observations were made on livers of successful parabiotic rats, at intervals during regenerative course of one of the partners which had been partially hepatectomized.

Changing pattern in size of hepatic cells was very similar between hepatectomized and non-hepatectomized partners. A considerable increase in number of mitotic figures and binucleate cells as well as in nuclear size, and some histochemical findings which were closely related to mitotic activity were observed only in the hepatectomized partner, as seen in partially hepatectomized single rats.

In conclusion, the compensatory hyperfunction of hepatic cells was evidenced in the non-hepatectomized partner after partial hepatectomy of the other, and therefore an exchange of humoral factor concerning stimulation of hepatic function was substantiated. While, the experiment yielded a negative result for the exchange of mitotic stimulating factor.

Many investigations have been made on hypertrophy, regeneration and proliferation of normal cells as well as on proliferation of tumor cells, but yet, many problems are remained unsolved on the subject. Experiments with partial hepatectomy as one of the ways to study the mechanism of hypertrophy, regeneration and proliferation of the cells have been made, and great many literatures on this subject such as those by Fishbach[26], Higgins and Anderson[37] and Brues[8][9] can be found. They used many kinds of animals such as rat, mouse, guinea pig, dog, rabbit etc. for the experiments.

The author has made histological, histochemical and micrometrical investigations on hepatic cells after partial hepatectomy of one partner of parabiotic rats in order to study on humoral factors controlling function and proliferation of the cells, as a part of series of the studies on senile changes by Tauchi *et al.*[57][83][86][89][99].

Several reports[11][21][38][40][45][46][73][96] on regeneration of the liver after partial hepatectomy of one partner of the parabionts are found in the literatures, and humoral exchange of so-called stimulating or inhibitory factors on mitosis has

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been discussed in them. But the number of the parabionts used and the duration of observation after partial hepatectomy were fairly limited in those experiments[11)21)38)40)96)], and they relied mainly on the number of mitotic figures. Moreover, there were many points of disagreement between these results. In regard to the size of the hepatic cells and their nuclei and the appearance of binucleate hepatic cells, Tauchi, Asamoto *et al.*[3)87)88)] discussed the relationship with the promotion and inhibition of cell division in their reports on senile changes of the hepatic cells. And regenerative process[99)] after hepatectomy in different age groups had been also observed in the close relationship with the change in volume of cells and nuclei, and in number of mitosis. The author would like to consider about the humoral factor in the regenerative process of the hepatic cells of parabiotic rats not only in the relationship with mitosis, but also with the changes in the volume of hepatic cells and nuclei, and in the histological and histochemical findings.

MATERIALS AND METHODS

A total of 144 inbred Wistar strain rats (88 ♀, 56 ♂) which were fed with the pellets (CA 1) supplied from the Central Laboratory of Experimental Animal, of 3 to 6 months of age and of 90 to 250 gs. of body weight were used in this experiment.

A pair of rats with approximate body weight and same sex were chosen among litter mates, and they were joined in parabiotic union by the modification of Bunster and Meyer's method[18)].

As a result of this operation, a total of 31 pairs (13 male pairs, 18 female pairs) were available as successful parabiotic rats[35)54)77)] in this experiment.

From 1 to 4 months after union, when they were 6-9 months old, the presence of cross circulation in the parabiotic rats was confirmed by visible excretion (within 20 minutes)[73)] of phenolsulfonphthalein (P.S.P.) in the urine of one partner of parabiont, after injection of 0.5 ml of 0.6% P.S.P. solution into gluteal muscle of the other. The midline incision was made in one partner of parabiont by Higgins and Anderson's method[37)] without anesthesia. After ligation was placed around the root of the anterior and left lateral lobes of the liver, these lobes were excised, removing about 65% of the liver[37)].

And the livers of both hepatectomized and non-hepatectomized partners were examined on sacrifice at 8, 18, 24, 30, 36, 48, 54, 60, 72, 96 and 120 hours after partial hepatectomy. Since there has been a report on diurnal difference in the amount of glycogen and in number of mitotic figures of the hepatic cells, the partial hepatectomy was performed so that the time of sacrifice falled between 11 A.M. and 2 P.M. The tissues were fixed in 10% formalin and absolute alcohol, embedded in paraffin, cut at 6 μ in thickness, and stained with hematoxylin-eosin, methylgreen-pyronin (stain of Unna-Pappenheim), and peri-

odic acid-Schiff (P.A.S.). Frozen sections fixed in formalin were stained with Sudan III.

Then, the changes with lapse of time were observed. Numbers of mitotic figures from metaphase to anaphase were counted with a magnification of 15 ×40 in the central and the peripheral zones of hepatic lobules in the removed lobes and the livers of both partners using paraffin sections stained with hematoxylin-eosin, and the number in 10,000 hepatic cells was used as the mitotic index.

Binucleate hepatic cells were also counted on the same H-E sections in each of the central and the peripheral zones, and the number in 10,000 cells was used for comparative study. The size of hepatic cells and their nuclei were measured on sections stained with H-E. In photomicrographs of the central and the peripheral zones, 100 hepatic parenchymal cells were chosen at randam, and their enlarged images were projected and drawn on the photographic paper of equal thickness. And then they were cut off along the outline of cells and nuclear membranes. After they were placed in the desicator for more than 24 hours to get rid of moisture from the papers, each of them was weighed with a Mettler analytical balance, and their weights were converted into the areas of hepatic cells and nuclei.

Square roots of these values of area were used for statistical analysis.

After total removal of the liver at the time of sacrifice, the body weight of each partner was measured after separating the parabiotic rats at the uniting line. Then the weights of the liver and the lost blood were added to obtain the correct body weight.

The values of weights of the livers of hepatectomized and non-hepatectomized partners were divided by the values of respective corrected body weights, to make the body-weight ratio.

RESULTS

The micrometrical values, histological and histochemical findings of hepatic parenchymal cells in the livers of both hepatectomized and non-hepatectomized partners and the initially removed lobes were summarized in Table 1 and 2.

The data on each study were plotted in the Fig. 1 to 15 with the lapse of time after partial hepatectomy as a horizontal axis.

1. The changes with time in mitotic index (Table 2 and Fig. 1)

There was usually seen no mitotic figure in 10,000 hepatic cells in the removed lobes in the resting stage.

In the liver of the hepatectomized partner, the mitotic figures were found scattered in both central and peripheral zones of the liver lobules, but more in the latter.

The mitotic index in the liver of the hepatectomized partner (Fig. 1) began

TABLE 1. Summary of Data by Various Measurements and of Histochemical Findings on Hepatic Cells of Hepatectomized and Non-Hepatectomized Partners of Parabionts

Hours after partial hepat.	No. of pairs			No. of binucleate liver cells (per 10,000 liver cells)	Size ($\sqrt{\text{area}}$ μ) Cells	Size ($\sqrt{\text{area}}$ μ) Nuclei	No. of giant nuclei (per 100 nuclei)	Wt. of the liver (g) / body wt.	RNA positive granules: Cells	RNA positive granules: Nucleoli	PAS positive granules	Sudan III positive granules
0	31	H	C	587± 23	15.37±0.19	5.89±0.32	0	1.34±0.05	++~+++	±~+	++~+++	−
			P	568± 23	15.36±0.18	5.97±0.17	rarely 1				++~+++	−
		N	C					3.83±0.14				
			P									
8~18	3	H	C	790±108	16.15±0.56	6.27±0.07	0.3±0.3	2.01±0.57	++~+++	+~++	±~+	+~++
			P	602± 6	16.64±0.36	6.52±0.09	1.3±0.9				±~+	++~+++
		N	C	622±138	15.87±0.54	6.29±0.16	0.7±0.7	4.09+0.56	++	±	++~+++	−
			P	583±129	16.39+0.16	6.23±0.38	0.7±0.7				++~+++	−
24~36	13	H	C	452± 38	16.44±0.30	6.37±0.15	2.6+1.1	2.74±0.17	++	++~+++	±~+	+
			P	489± 36	17.38±0.29	6.99±0.15	8.9±3.0				±~+	++~+++
		N	C	563± 40	16.34±0.27	6.08±0.11	0.8±0.4	4.15±0.18	++~+++	±~+	++~+++	−
			P	533± 55	16.64±0.36	6.27±0.10	0.3±0.2				++~+++	−
48~60	9	H	C	543± 41	17.32++0.35	6.82±0.21	5.9+3.0	3.08±0.16	++~+++	++	+	±
			P	472++ 35	17.83++0.26	7.29±0.26	19.4±4.7				+	++
		N	C	610± 65	16.50±0.29	6.23±0.12	0.2±0.1	4.30±0.15	++	+~++	++~+++	−
			P	606± 54	16.40±0.23	6.19±0.13	0.6±0.4				++~+++	−
72	2	H	C	558±193	16.72±0.35	6.40±0.05	1.0± 0.01	3.16±0.54	++~+++	++	+~++	−~±
			P	503±103	17.83±0.23	6.99±0.57	2.0±11.0				++~+++	+~++
		N	C	753±183	16.20±0.96	6.23±0.20	0.5±0.5	4.26±1.30	++~+++	+~++	++~+++	−
			P	710±115	15.63±0.10	6.30±0.03	0.5±0.5				++	−
96	2	H	C	608±188	16.15±0.02	6.39±0.10	0.5±0.5	3.21±0.53	++	+~++	−~±	−
			P	470±160	16.64±0.19	7.22±0.10	13.5±0.3				−~±	+
		N	C	683± 58	15.08±0.19	5.90±0.14	0	3.68±1.03	+++	+	±	−
			P	623± 58	15.51±0.19	6.32±0.19	0.5±0.5				+	−
120	2	H	C	553± 93	16.45±0.57	6.71±0.17	6.0±5.0	3.07±0.69	++	+~++	±	±~+
			P	508±138	16.50±0.59	7.17±0.01	20.5±0.3				±~+	++
		N	C	663± 13	15.05±0.04	6,06±0.02	0	3.91±0.12	++~+++	±~+	±~+	−
			P	552±108	14.56±0.29	6 00±0.33	0				+	−

Note: H: Hepatectomized partner
N: Non-hepatectomized partner
C: Central zone
P: Peripheral zone

to increase 24 hrs. after the operation, reached a peak of mitotic activity from 30 to 36 hours, and then gradually decreased after 48 hours; but a considerable number of mitotic figures were still found at 120 hours. In the liver of the non-hepatectomized partner, mitotic figures did not increase significantly except

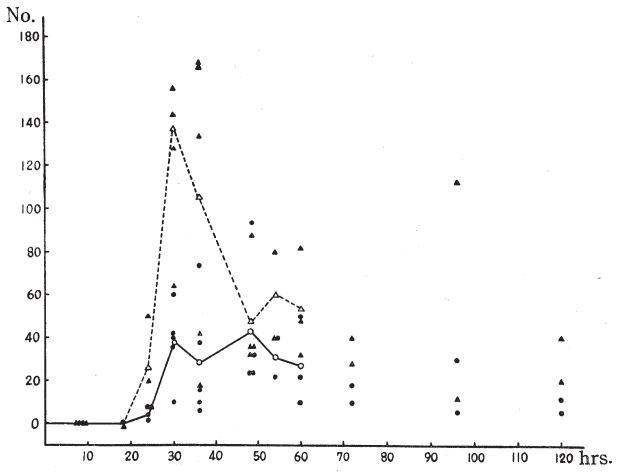

FIG. 1. Change in mitotic indices in hepatectomized partners.
● Central zone ▲ Peripheral zone

TABLE 2. Mitotic Indices in Livers of Hepatectomized and Non-Hepatectomized Partners of Parabionts

Hours after partial hepat.	No. of pairs	Hepatectomized		Non-hepatectomized	
		No. of mitotic figures in 10,000 hepatic parenchymal cells			
		Central zone	Peripheral zone	Central zone	Peripheral zone
0	31	0, rarely 1 or 2			
8	2	0	0	0	0
18	1	0	0	0.91	0.31
24	3	4.66± 1.76	26.00±12.49	0.32±0.31	0.43±0.42
30	5	37.60± 8.03	137.60±21.45	1.43±0.49	1.20±0.45
36	5	28.80±13.03	105.60±31.71	0.19±0.18	0.68±0.18
48	4	43.50±16.76	48.00±13.36	0	0
54	2	31.00± 9.00	60.00±20.00	1.46±1.46	1.32±1.32
60	3	27.33±11.90	54.00±14.80	0.42±0.42	0.25±0.12
72	2	14.00± 4.00	34.00± 6.00	0.67±0.67	0.23±0.23
96	2	18.00±12.00	62.00±50.00	1.23±1.23	0.11±0.11
120	2	9.00± 3.00	30.00±10.00	0.37±0.37	0.27±0.01

for a few cases.

2. The changes with time in the number of binucleate hepatic cells (Fig. 2, 3)

The average numbers of binucleate cells in 10,000 hepatic cells were 586.6 ±22.8 in the central zone and 568.4±22.7 in the peripheral zone of the lobules in 31 specimens of removed lobes. In the liver of the hepatectomized partner, its number was increased soon after the operation, and then reduced to the lowest level during the 30–36 hours, when the mitotic activity was maximum.

In the liver of the non-hepatectomized partner, the individual differences were

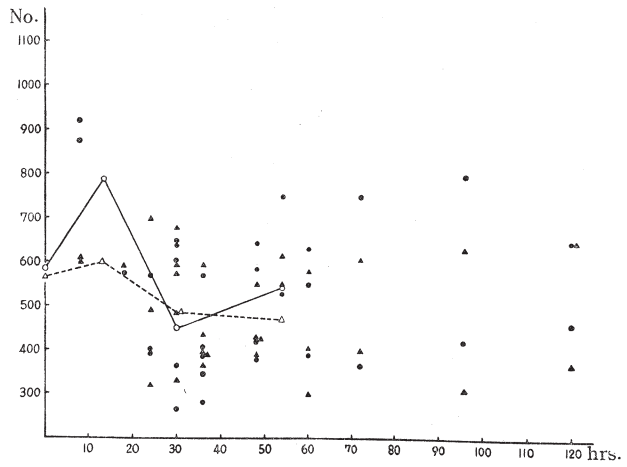

FIG. 2. Change in number of binucleate cells in hepatectomized partners
● Central zone ▲ Peripheral zone

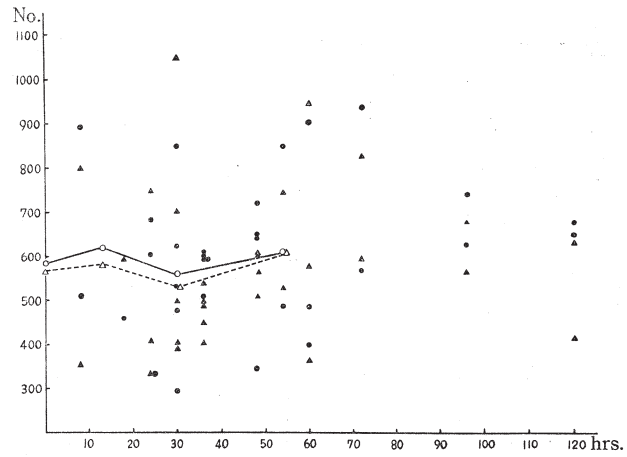

FIG. 3. Change in number of binucleate cells in non-hepatectomized partners
● Central zone ▲ Peripheral zone

great in the appearance of binucleate hepatic cells and any significant change in the number of the cells was found in earlier stage after hepatectomy.

3. The changes with time in the histochemical findings

The post hepatectomy changes in RNA granules in the hepatic cells and nucleoli, in PAS positive granules and in Sudan III positive granules were shown in Fig. 4 to 8.

a. RNA granules in the hepatic cells (Table 1)

The RNA was seen in the hepatic cells as pyronin positive granules which

varied from very fine to coarse.

The granules were coarse in the cells which were dense in them, and were fine in the cells which are scarce.

There was almost no difference in the appearance of the granules between the central and the peripheral zones of hepatic lobules. There was some variation in the appearance of the granules between individual cells.

No remarkable fluctuation was observed in the hepatic RNA granules in either partner throughout the experiment. A slight, if any, increase was noted in the liver of the hepatectomized partner.

b. RNA granules in the nucleoli (Fig. 4, 5)

The RNA in the nuclei was present mainly in the nucleoli, though there was individual cell variation in its appearance.

Its general tendency was that the RNA in the nucleoli of the liver of the hepatectomized partner began to increase soon after the partial hepatectomy, and reached a peak during 30–36 hours, then decreased gradually. But it was still higher than the control value at 120 hours after operation.

In the liver of the non-hepatectomized partner, it was increased only slightly

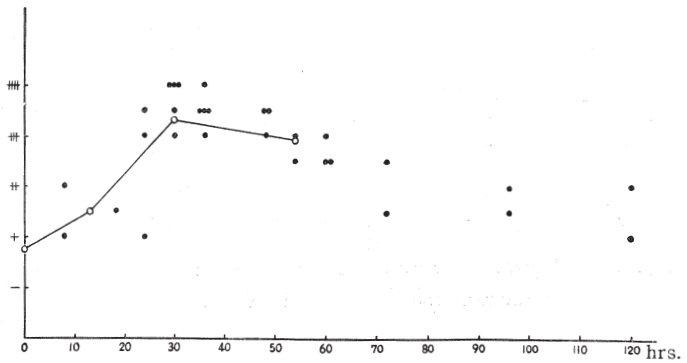

FIG. 4. Change in RNA granules in nucleoli in hepatectomized partners

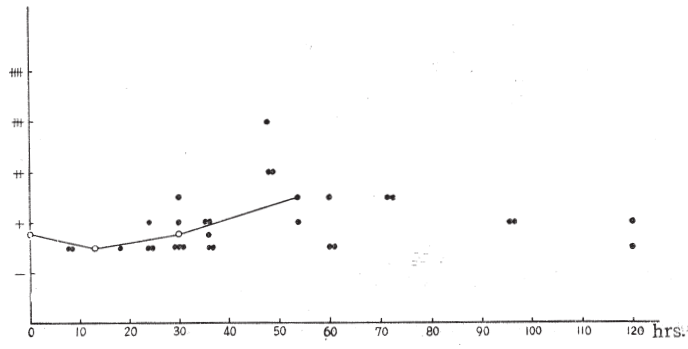

FIG. 5. Change in RNA granules in nucleoli in non-hepatectomized partners

during 48–72 hours.

c. PAS positive granules (Fig. 6, 7)

PAS positive granules were seen as coarse granules in the hepatic parenchymal cells. In the excised lobes, they were, in general, stained almost filling up the cells. It appeared that they were rather more dense in the peripheral zone of lobules than in the center, though the difference was not very marked.

PAS positive granules in the liver of the hepatectomized partner were decreased rapidly after hepatectomy, and showed fewer number than in the excised portion of the liver throughout the experiment.

In the liver of the non-hepatectomized partner, the PAS positive granules showed a temporary slight increase soon after the operation, but were reduced and kept at the level lower than that of the excised portion during 30–72 hours after the operation, followed by a gradual decrease up to 120th hour.

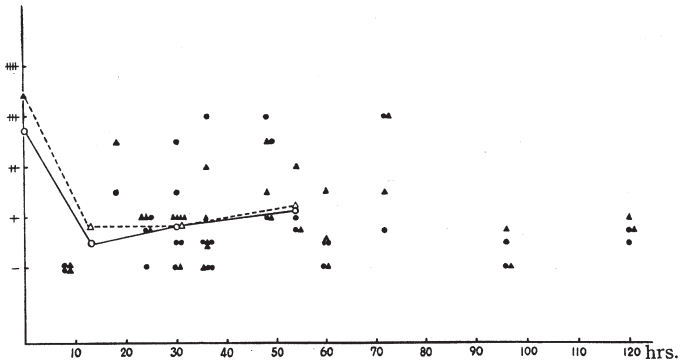

FIG. 6. Change in PAS positive granules in cytoplasm in hepatectomized partners
● Central zone ▲ Peripheral zone

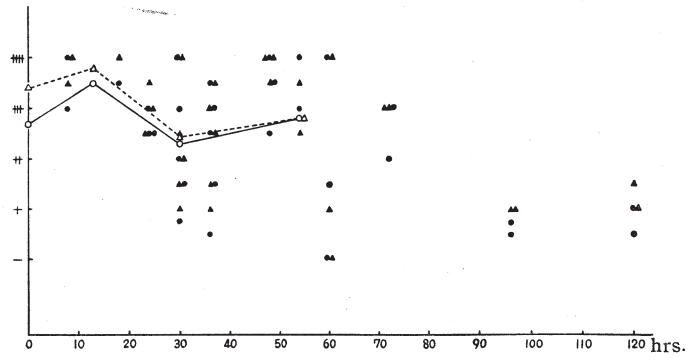

FIG. 7. Change in PAS positive granules in cytoplasm in non-hepatectomized partners
● Central zone ▲ Peripheral zone

d. Sudan III positive granules (Fig. 8)

Sudan III positive granules in the liver of the hepatectomized partner began to appear in the early stage of post hepatectomy, and reached the maximum during 24–48 hours, then decreased gradually. But a considerable number of Sudan III positive granules were still found at 120 hour.

They were found in the liver lobules scattered irregularly, but generally more in the peripherel zone.

Most cells contained more than 3-4 comparatively fine granules, but occasional cells contained fewer and larger droplets. Degenerative changes of the nuclei were not found.

In the liver of the non-hepatectomized partner, the Sudan III positive granules were not seen throughout the experiment.

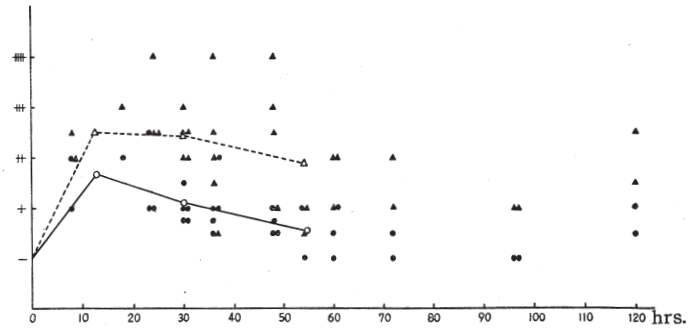

FIG. 8. Change in Sudan III positive granules in cytoplasm in hepatectomized partners
● Central zone ▲ Peripheral zone

5. The sizes of the hepatic parenchymal cells and of their nuclei

Using the square root of the areas of hepatic cells and their nuclei, the values in the livers of hepatectomized and non-hepatectomized partners were compared with the values in the excised livers. Then the differences were plotted with the lapse of time after partial hepatectomy.

a. The change in the cell size (Fig. 9, 10)

In the liver of the hepatectomized partner, the size of hepatic cell began to increase 24 hours after the operation, and reached the maximum during 30–54 hours. The increase was statistically significant ($P<0.01$) during 48–54 hours in both central and peripheral zones. And then it gradually decreased, but showed still a slightly larger value than the control value of excised portion 120 hours after the hepatectomy.

In the non-hepatectomized partner, the change of the cell size was similar but slightly smaller than that in the hepatectomized one. The increase was also statistically significant ($P<0.01$) during 48–54 hours in both zones. The increase was generally a little more marked in the peripheral than in the

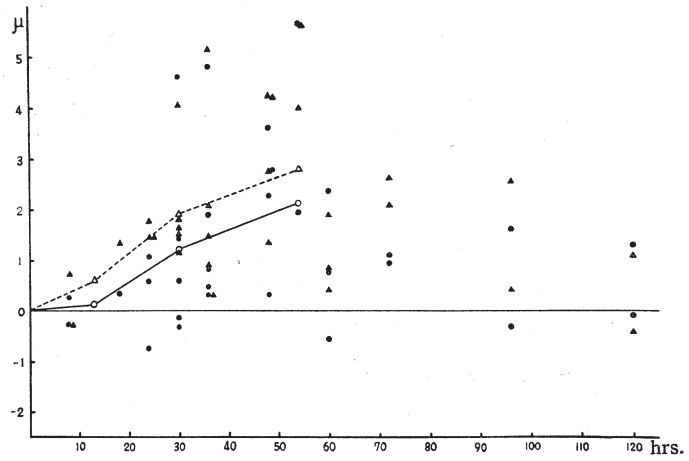

FIG. 9. Change in size of cells ($\sqrt{\text{area in hepatectomized}}-\sqrt{\text{area in excised}}$) in hepatectomized partners
● Central zone ▲ Peripheral zone

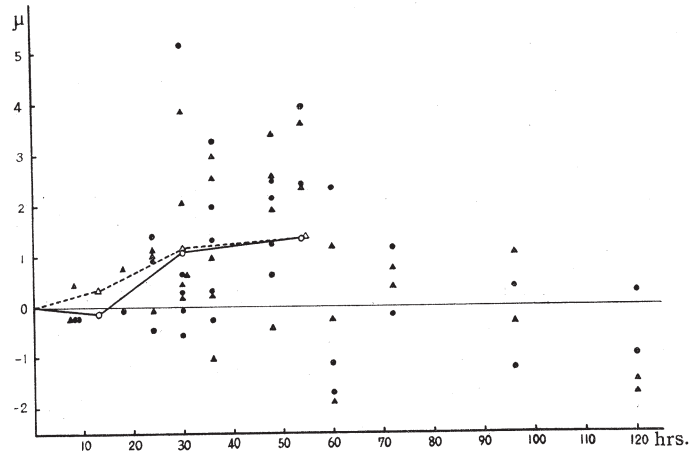

FIG. 10. Change in size of cells ($\sqrt{\text{area in non-hepatectomized}}-\sqrt{\text{area in excised}}$) in non-hepatectomized partners
● Central zone ▲ Peripheral zone

central zone.

b. The change in the nuclear size (Fig. 11, 12)

In the liver of the hepatectomized partner, the nuclei of parenchymal cells began to increase their volume in the early stage of post hepatectomy, reached the maximum during 30–54 hours later, and showed an increase with a statistically significant difference ($P<0.05$) during 48–54 hours in both zones. Then the nuclear size was decreased slightly at 72 hours but still maintained

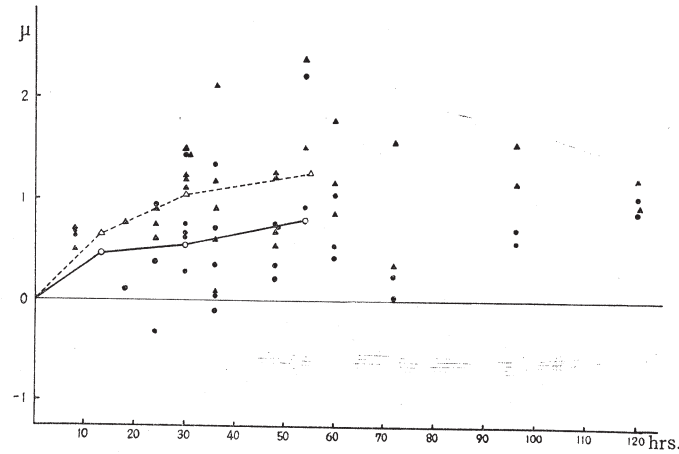

FIG. 11. Change in size of nuclei ($\sqrt{\text{area in hepatectomized}}-\sqrt{\text{area in excised}}$) in hepatectomized partners
● Central zone ▲ Peripheral zone

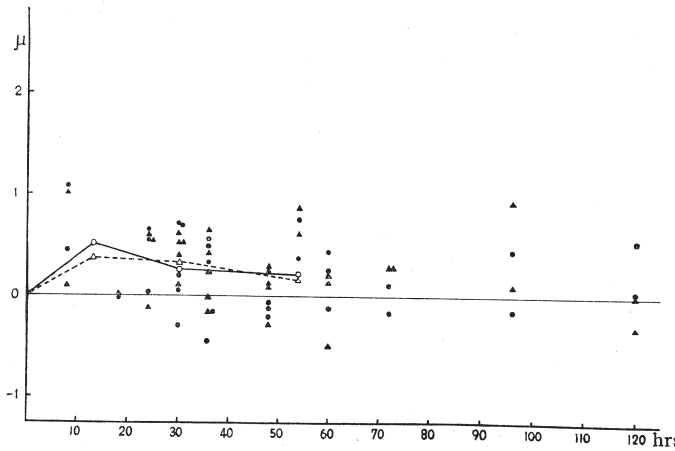

FIG. 12. Change in size of nuclei ($\sqrt{\text{area in non-hepatectomized}}-\sqrt{\text{area in excised}}$) in non-hepatectomized partners
● Central zone ▲ Peripheral zone

a remarkable increase 120 hours after the operation.

In the liver of the non-hepatectomized partner, the increase in nuclear size was very slight, compared with that in the hepatectomized. The nuclear size seemed to be slightly increased from the early stage, but it did not show any significant difference in either zone during 48–54 hours. And so, throughout all the lapse, it had not shown such a remarkable change.

c. Appearance of giant nuclear cells and the irregularity of the nuclear size (Fig. 13, 14)

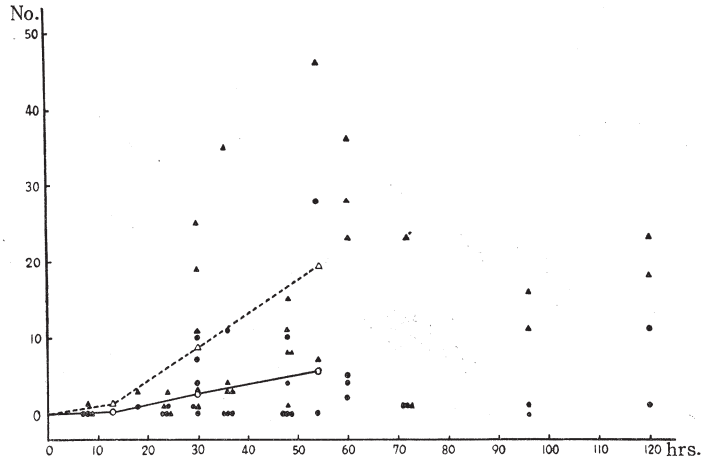

FIG. 13. Change in number of cells with giant nuclei in hepatectomized partners
● Central zone ▲ Peripheral zone

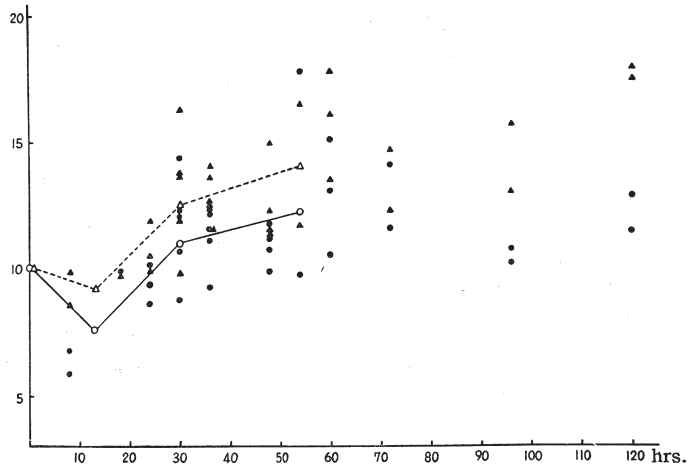

FIG. 14. Change in coefficiency of variation of size of nuclei in hepatectomized partners
● Central zone ▲ Peripheral zone

In this project, the nucleus whose area was more than two times as large as the mean value of the size of hepatic cell nuclei in the removed lobes was considered to be a giant nucleus and the number of cells with giant nucleus was counted in 100 hepatic parenchymal cells. The irregularity of the nuclear size was indicated by its coefficiency of variation.

The giant nuclei were rarely found in the excised portions of the liver.

The liver of the non-hepatectomized partner showed only a slight tendency of increase in the number of giant nuclei, when compared with excised liver.

In the liver of the hepatectomized partner, the number of the cells with giant

nuclei began to show an obvious increase after partial hepatectomy, reached maximum at about 54 hours, then after a temporary slight decrease, they were gradually increased till 120 hours after operation.

The irregularity of the nuclear size showed a temporary and slight decrease in the early stage of post hepatectomy, but soon increased definitely in the liver of the hepatectomized partner. It showed almost similar tendency with the change of the nuclear size and of the appearance of the giant nuclear cells. But hardly any change was observed in the irregularity of the nuclear size in the non-hepatectomized partner.

6. The change in the liver weight (Fig. 15)

In the hepatectomized partner, the weight of the residual liver was rapidly increased after operation, doubling the weight at 30 hours.

The weight continued to be increased slowly thereafter up to 120 hours after the operation, at which time the liver did not regain the initial weight.

The liver of the non-hepatectomized partner showed a very slow increase in weight till 54 hours after operation, maintained the weight till 72 hours, then began to decrease. The liver returned to the initial weight at 120 hours.

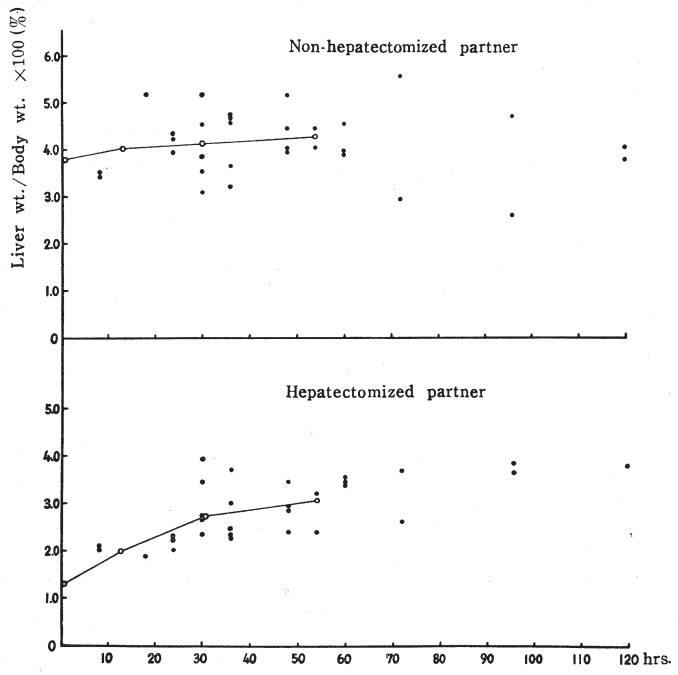

FIG. 15. Changes in weight of livers in parabiotic rats after partial hepatectomy in one partner

DISCUSSION

Hypertrophy, regeneration and proliferation of the liver tissue have been studied and discussed in details by several authors.

Mitotic figure was rarely seen in normal hepatic cells of adult rats[8)30)67)91)] and there were few mitoses in the cells of the resting stage of 6–9 months old parabiotic rats used in this experiment. In regard to the relation between mitosis and its localization within the lobules different results have been reported by various investigators and on various species of animals[23)41)56)58)81)99)].

As in Yamada's report[99)], more mitotic figures were found in the peripheral zone in the liver of the hepatectomized parabiotic rat in this study.

The maximal incidence of mitosis after partial hepatectomy on rats was shown by many investigators that the peak was reached at 24th hour[1)9)], 28th or 29th hr.[95)], 30th hr.[80)99)], 36th hr.[34)] 48th hr.[37)69)] or 72th hr.[98)100)]. It may be considered that these differences are due to differences in age, nutritional condition and strain of animals.

In any case, active proliferation of hepatic cells occurs in earlier stage of post partial hepatectomy. But there are still many opinions in regard to a stimulating[30)31)72)] or an inhibiting factor[31)75)] directly governing hepatic regeneration.

Two factors have been mainly considered as fundamental to cause liver regeneration in a single rat after partial hepatectomy.

One factor is the change of portal circulation, another is the change of concentration of blood protein. Since interest was focused on the portal vein by Rous and Larimore[74)] who showed that ligation of a branch of the portal vein in rabbits led to atrophy of the lobe supplied by it and to hypertrophy of the rest of the liver, the blood supply of the liver has for long been considered to play a fundamental role in the control of regeneration. Its importance in regeneration has been emphasized by Mann[36)48)49)50)78)]. But Karn and Vars[44)] later reported that ligation of the hepatic artery did not affect regeneration of the liver in rats, and Weinbren[94)] showed that regeneration did in fact occur in the absence of a portal blood supply in rats. It was also reported that if the normal portal blood supply is replaced by the arterial or inferior vena cava supply, regeneration proceeded normally[20)27)28)].

In any case it is difficult to consider that gross change of portal blood flow would occur in the liver of the non-hepatectomized partner. On the other hand, Glinos and Gey[31)] found that plasmapheresis caused mitosis in the livers of normal rats, and Glinos[32)33)] had produced evidence that, conversely, the regenerative changes after partial hepatectomy can be inhibited by increasing plasma concentration and that the active fraction is the albumin. On the basis of these findings, he suggested that plasma albumin concentration controls regeneration.

Besides, McJunkin and Breuhaus[55] found a higher incidence of mitotic figures in the liver remnant when necrotic liver tissue was present in the peritoneal cavity.

Furthermore there are many factors influencing hepatic regeneration after partial hepatectomy; such as nutritional[4)9)24)53)59)71)], age[7)10)52)61)], hormonal[19)21)39)82)], and diurnal differences[42)86)]. These problems had been discussed in detail by each worker.

On the other hand, there are many reports concerning the mitosis stimulating factor on the normal hepatic cells; Teir and Ravanti[91], and McJunkin and Breuhaus[55] obtained a small number of mitosis in normal liver by intraperitoneal injections of pulped liver. Marshak and Walker[51] also produced a few mitoses by the intravenous injection of liver nuclei and various fractions of them.

Wilson and Leduc[97] reported that the mitotic activity in the livers of young mice is inhibited at first and stimulated later by the intraperitoneal injection of pulped liver of mouse and guinea pig, by boiled or autolyzed liver, pulped kidney, and by boiled egg yolk. It has been known that many substances are capable of stimulating a mitotic activity in the liver by injection into animals. Thus trypan blue, trypaflavine, thiourea, thyroxin, colchicine and staphylococcus toxin are all capable of producing mitotic figures in the liver of animals without evidence of parenchymal damage[67)70)]. Paget[67] considered that the mitotic effect with some of the chemical agents mentioned is a "dilution proliferation", since cell proliferation may be regarded as a defence mechanism against the accumulation of toxic substances in the cell. The reports by Christensen and Jacobsen[21], Bucher, Scott and Aub[11], Wenneker and Sussman[96], and by Kitani *et al.*[45)46)] on the increased mitotic activity 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".

On the other hand, Islami *et al.*[40] showed that the rate of mitosis in the livers of the intact parabiotic partners was at most equal to or even slightly less than the rate in the livers of normal individual controls. Rogers[73] *et al.* investigated regeneration of the liver in single and parabiotic paired and "triplet" rats following sham operation and partial hepatectomy. He found that partial hepatectomy in parabiotic paired and triplet rats produced in the operated liver a regenerative responses which was similar to that observed in partially hepatectomized single rats, and failed to elicit a statistically significant response in mitosis in a non-hepatectomized partner.

From these results, they did not support the conclusion that the blood in parabiotic rats carries stimulating of inhibitory "humoral factors" directly governing hepatic regeneration.

But when these reports are compared and examined, it must be questioned

if the state of commingling circulation of blood is similar.

Fleming *et al.*[29] employed P^{32} tagged erythrocytes for measurement of blood exchange between parabiotic rats. And the value obtained was 2, 5 times as much as that obtained by Van Dyke *et al.*[93] who used Fe^{59} in their investigation. He considered their differences to be due to the factors of age of animals and duration after parabiotic operation. Rates of cross circulation of erythrocytes and plasma in successful and intoxicated parabionts were determined simultaneously with $NaCr^{51}O_4$ tagged erythrocytes and with radio iodinated human serum albumin and the results indicated profound slowing of circulation in intoxicated parabionts, and also normal pairs reinvestigated 10 and 15 months after surgical union showed some slowing of cross circulation[6].

Kamrin[43] showed that in the serial study of the A/G ratios in successful parabiotic pairs, A/G ratios of the pairs were almost alike on 27th and on 35th day following surgical union.

Therefore, it is desirable to employ successful parabionts with completely healed wound and of more than one month old after operation, in the study of mitotic stimulating humoral factor.

In this study, successful parabionts of 1 month or more old after parabiotic operation were used.

The resting stage of the liver in these parabionts showed some histological differences with the liver of the control single rats. On this respect, there are reports by Sauerbruch[76] and others[5)25)60)77]. It may be considered that those histological changes are due to toxic substances produced as a result of antigen-antibody reaction in parabionts. In this study, however, mitosis did not show a definite increase in a non-hepatectomized partner whereas the mitotic activity in a hepatectomized partner was almost identical with that in the liver of a partially hepatectomized single rat reported by Yamada[99] and others.

This observation differs from the results by Christensen and Jacobson[21], Bucher, Scott and Aub[11], Wenneker and Sussman[96] and Kitani *et al.*[45)46)] and is almost agreeable with the reports by Islami[40], and Rogers[73].

There are some different views upon the significance of binucleate hepatic cells. In general, binucleate cells have been considered to be produced by amitosis[22)23)], and can be seen more frequently in the peripheral zone in the rabbit liver[68]. They are increased with aging in the human liver[56)90)] and are thought to be caused by amitosis in unfavourable conditions[63)64)65)66)]. It is reported that the increase in binucleate cells in regenerating livers after partial hepatectomy precedes the peak of mitotic activity, and they are gradually decreased with advancing mitotic activity[34)98)].

Bucher[12] considered that amitosis has its significance in increase of number of nuclei, and not in cell proliferation.

It is interesting that binucleate cells are increased in older animals with a declining regenerative function[89].

In this experiment, binucleate hepatic cells are increased in the hepatectomized partner in earlier stage after partial hepatectomy, then gradually decreased while mitosis is active, and more of them are found in the central zone than in the peripheral zone, as has been observed in the liver of partially hepatectomized single rats[85].

On the other hand, the findings are rather inconsistent in the non-hepatectomized partner.

There are many reports in regard to the relationship between mitosis and RNA in nucleolus and cytoplasm. Since the study of Caspersson *et al.*, it has been confirmed by many workers that when RNA is abundant in cytoplasm and nucleolus, protein synthesis in the cell is very active[62]. And there are many reports[52)62)69)101)] concerning an increase of RNA in cytoplasm and nucleolus in hepatic cells after partial hepatectomy, but there is no decided agreement in correlating mechanism between mitosis and RNA. It has been considered that RNA in hepatic cell cytoplasm is present largely in microsome and the prevailing opinion at present is that RNA synthesis is accelerated by the same mechanism that promotes the protein synthesis rather than that the former plays a direct role in the latter.

In this experiment, there were no differences between the hepatectomized and non-hepatectomized partners in the content and changing pattern of RNA in hepatic cell cytoplasm.

On the other hand, RNA in hepatic cell nucleoli in the hepatectomized partner was obviously increased after the operation, and changed parallel with mitotic activity. But the RNA in nucleoli in the non-hepatectomized partner showed a very slight increase following the peak of mitotic activity.

There are many reports on mitosis and PAS positive granules especially glycogen, such as those by Bullough[13)14)15)16)17)], Jaffe[42)], and Tauchi *et al.*[86)]. Glycogen in the liver of the hepatectomized partner was showed to be decreased rapidly after partial hepatectomy, being lowest in the active stage of mitosis, as in single rats after partial hepatectomy. In the non-hepatectomized partner, the glycogen was showed to be increased a little in the early stage of partial hepatectomy, then decreased gradually, and there was no difference between both partners 72 hours after partial hepatectomy.

In spite of many reports[4)46)47)92)99)101)] on the appearance of Sudan III positive granules in the hepatic cell of post partial hepatectomy, there are many unknown factors about the significance of the appearance.

In the liver of the hepatectomized partner, Sudan III positive granules began to appear in the early stage of postoperation, and showed the highest level at the peak of mitosis, seen more in peripheral zone throughout postoperation period as in single rats after partial hepatectomy. But there was none to appear in the liver of the non-hepatectomized partner in the author's experiment.

In regard to change in the volume of liver cells and of their nuclei after

hepatectomy, there are reports by Brues[8], Yoshinaga[101], Stowell[79][80], Suga[81], and Yamada[99], all of which showed an increase in the volume of the cells and nuclei before the onset of mitotic activity after partial hepatectomy.

Stowell[80] considered that the increase in their volume before the stage of mitosis was caused by water absorption as a preliminary step for protein synthesis, and by the appearance of Sudan III positive granules. Suga[81] studied on the hepatectomy of starved rabbits, and Yamada[99] showed that the increase in the volume was more marked in the senile rats showing inactive mitosis. Both considered that the increase in the volume was compensatory temporary hypertrophy for the loss of hepatic cells caused by partial hepatectomy.

Tauchi[84] and Morikawa[57] also reported, that increases in the volume of hepatic cells and their nuclei in the old human were compensatory hypertrophy for the decreased number of hepatic cells caused by decreased regenerative function. As for relationship between cell proliferation and hypertrophy, it was suggested by Adolph[2] that increases of cell numbers and of cell contents are independent to each other. And Brues[9] expressed his opinion that the increase of cellular and nuclear volume and mitosis were independent to each other, because mitosis can be induced without increase in the cellular volume by partial hepatectomy in the rats starved for 3-4 days before the operation.

In this experiment using parabiotic rats, the size of hepatic cells and nuclei in the hepatectomized partner shows an initial increase before the appearance of mitotic figures, and shows the highest value in the somewhat later stage than the peak of mitotic activity with a significant ($P<0.05$) increase in both central and peripheral zones during 48-54 hours after operation.

But the size of hepatic cells in the non-hepatectomized partner also increases significantly as in the hepatectomized during 48-54 hours after operation, while the size of nuclei in the non-hepatectomized partner shows no statistically significant change, though a slight tendency of increase is seen.

Several reports concerned with the appearance of giant nuclear cells and irregularity in size of nuclei are also found in the literature.

Tauchi *et al.*[3][87][88] reported that the irregularity in size of nuclei and appearance of giant nuclei of hepatic cells are morphological expression of an active state toward cell division in the inhibitory condition. Grundmann[34] observed regeneration of the liver for 54 hours after 2/3 hepatectomy and measured nuclear volumes and DNA content. He found a gradual increase in number of giant nuclei as a result of polyploidy formation due to endomitosis which, he thought, regulated the function of the cells together with appearance of binucleate hepatic cells. The results of this experiment are summarized in the following sentences:

The common changes in the livers of both partners are a significant increase in size of hepatic cells and the appearance of RNA in hepatic cells.

The findings on binucleate hepatic cells in the hepatectomized partner were

similar to those in the hepatectomized single rats, but they were inconsistent in the non-hepatectomized.

Increases in the nuclear volume, RNA in nucleoli, mitotic activity, and Sudan III positive granules and a decrease in PAS positive granules in the liver of the partially hepatectomized partner of parabionts were very similar to the changes in the liver of partially hepatectomized single rats. But the liver of the non-hepatectomized partner showed no increase in Sudan III positive granules and only a gradual decrease in PAS positive substance.

It can, therefore, be said that mitosis and its closely related phenomena are similarly observed in the livers of the hepatectomized partner of parabionts and of hepatectomized single rats, but not seen in the non-hepatectomized partner. On the other hand, it seemed that the hypertrophic change of the hepatic cells was very similar in both hepatectomized and non-hepatectomized partners of parabionts. This experiment indicates that the liver of the non-hepatectomized partner compensates the functional insufficiency of residual liver of the hepatectomized, and therefore is a favorable evidence for humoral interchanges between partners of parabionts.

It is interesting that there is no interchange of mitosis-stimulating factor, in spite of the fact that there is humoral interchange of function stimulating factor.

This experiment therefore would give a base for the opinion of Mann *et al.*[36)48)49)50)78)] that mitoses after hepatectomy are induced by the change of portal circulation. On the other hand, it is also considered that only so-called functional factor of the hepatic cell is permitted to interchange, but not the proliferative factor concerning mitosis in the parabiotic rats used by the author, if the mitosis is induced by changes in blood component as in the opinion of Glinos *et al.*[31)32)].

The latter reasoning would give a support to the opinion of Adolph[2)] and Brues[9)] that hypertrophy and proliferation of the cell are essentially independent to each other. The observed period after partial hepatectomy in this experiment is limitted and, of course, this experiment is not designed to clarify the whole mechanism of cell proliferation. But the results of this experiment would contribute considerably to the solution of the mechanism.

SUMMARY AND CONCLUSION

Rats of Wistar strain of 3-6 months old were paired in order to investigate on the regeneration and proliferation of the cell. One partner of the parabiont was partially (65%) hepatectomized, when 1 month or more have passed after the union, and histological, histochemical and micrometrical observations on the hepatic cells were made along the course of time from 8 hrs. to 120 hrs. after operation in both hepatectomized and non-hepatectomized

partners comparing to partially hepatectomized single rats.

1. In regard to the mitotic activity after hepatectomy, the liver of the hepatectomized partner of the parabionts showed a similar tendency to that in the liver of hepatectomized single rats. And the liver of the non-hepatectomized partner did not show any significant difference from the intact liver of single rats.

2. Binucleate hepatic cells were increased in the early stage of postoperation in the operated partner, but not in the non-hepatectomized one.

3. The change in RNA in nucleoli in the operated partner was almost similar to that in the residual liver of the single rat, and the liver of the non-hepatectomized partner showed a very slight, if any, increase in RNA in nucleoli after the peak of mitotic activity.

The appearance of Sudan III positive granules in the liver of the hepatectomized partner was similar to that in the residual liver of single rats, but they did not appear in the liver in the non-hepatectomized partner at any stage after the operation. The PAS positive granules in the liver of the hepatectomized partner were decreased rapidly in the early stage of post operation, but they were not decreased in the liver of the non-hepatectomized partner.

4. The size of the hepatic cells was similarly increased in both partners with the time after hepatectomy.

The size of the nuclei in the hepatectomized partner was markedly increased after partial hepatectomy, showing a similar pattern of change with the size of the hepatic cells. But in the non-hepatectomized partner, there was only a slight tendency of increase in the nuclear size.

The number of the giant nuclear hepatic cells was increased obviously paralleling with the change in size of hepatic nuclei. There was scarce increase in the giant nuclei in the non-hepatectomized partner.

5. The weight of the residual liver in the hepatectomized partner was increased fairly rapidly until the peak of mitotic activity, and then slightly thereafter. But a complete recovery of the initial weight was not attained even at 120 hrs. after hepatectomy. Liver weight of the non-hepatectomized partner was slightly increased being almost parallel with the increase in size of hepatic cells until 72 hrs. of posthepatectomy, and then it was rather decreased.

6. In conclusion the compensatory hyperfunction of hepatic cells was evidenced in the non-hepatectomized partner of the parabiotic rats after partial hepatectomy of the other, and therefore an exchange of humoral factor concerning stimulation of hepatic function was substantiated. However, the experiment was rather negative for the exchange of mitotic stimulating factor.

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