tration of intoxicating doses of alcohol had no effect on the concentration of GABA in the hindbrain but produced small decreases in the concentrations of glutamate and aspartate.

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Lysosomal Enzymes in Regenerating Rat Liver* (33844)

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The possible role of the lysosomes or certain lysosomal enzymes in the process of cell division has been described by many authors. Adams (2) showed that acid phosphatase and acid DNase attain their highest concentration in regenerating rat liver about 3–5 hr before the DNA synthesis of the cell is at its maximum. Allison and Malluchi (4) observed that the lysosomes in phytohemagglutinin-stimulated lymphocytes were enlarged and considered this a primary phenomenon associated with the initiation of cell mitosis. The location of the lysosomes at various stages of cell division has been studied electron microscopically by Robbins and Gonatas (16) who observed acid phosphatase-positive particles during the prophase. During the metaphase this phenomenon was even more conspicuous. Kent *et al.* (14) reported that iron-laden lysosomes were localized according to a certain pattern during the early stages of mitosis.

During the first 24 hr of regeneration, minimal or no biochemical variations in the activity of free and total acid phosphatase have been described (20). The same applied to acid phosphatase and acid RNase during the first 8 days of regeneration (3).

Material and Methods. Rats of the Sprague-Dawley strain, weighing 200–300 g, were used. Hepatectomy was carried out by the method of Higgins and Andersson (12), about 70% of the liver was removed. The operation was performed at the same time of the day in order to eliminate the influence of

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the mitotic 24-hr variations in regeneration. The liver lobes were removed under ether anesthesia and under sterile conditions.

The regenerating liver was collected after 1, 2, 3, and 10 days. "Normal" untreated liver (0 days) was obtained from intact rats, and tissue obtained at hepatectomy represented "normal manipulated" liver. In addition, in one group of animals sham operation was performed and specimens were collected 1 day later.

The samples were suspended in 0.25 *M* +4° saccharose in the proportion 1:10 and homogenized with a motor-driven Taflon pestle at about 2000 rpm three times up and down. The liver homogenate was fractionated by the technique described by Savant *et al.* (18) *e.g.*, centrifuged at 750*g* for 10 min and 3300*g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 16,-300*g* for 10 min. The pellet = fraction F I and supernatant were used for enzyme determinations. Samples were taken from the homogenate, fraction F I and its supernatant.

The determination of acid phosphatase was performed with β-glycerophasphate as substrate in 0.1 *M* acetate buffer at a pH of 5.0 and with 15-min incubation (6). The amount of phosphorus released was determined according to Fiske–Subbarow (9). Arylsulfatase B was determined according to Roy (17) at a pH of 5.7 using 2-*OH*-4-nitrocatecholsulfate dipotassium salt (Sigma) as substrate. The time of incubation was 60 min. Only the total enzymatic activity was noted, since the lysosomal membranes burst owing to the protracted incubation. The β-glucuronidase activity was determined with phenolphthalein glucuronide chinchonidine salt (Sigma) as substrate at a pH of 5.2 in 0.1 *M* acetate buffer after 20-imn incubation at 37° (8). The effect of 0.25 *M* saccharose can be excluded (10), since the method has been standardized.

For determination of the "total" enzymatic activity, 0.1–0.2% Triton X-100 was added to the incubation medium. All assays were made in duplicate and O-tests were performed. Protein determination was carried out according to Lowry *et al.* (15).

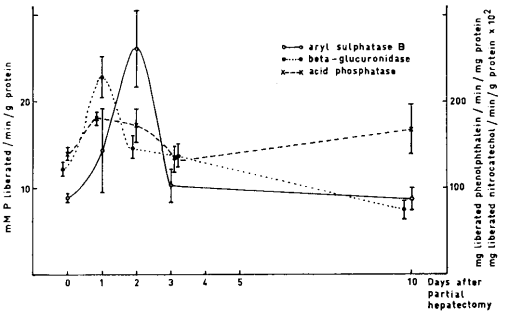

FIG. 1. The total specific activity of lysosomal enzymes in the homogenate of regenerating liver ± SE.

Specific activity is expressed as follows: Acid phosphatase, mmoles of liberated P/min/g of protein; β-glucuronidase, mg of liberated phenolphtalein/min/mg of protein; arylsulfatase B, mg of liberated nitrocatechol/min/mg of protein. The total activity is obtained by multiplying the specific activity by the total amount of protein in the sample.

Results. The distribution of the lysosomal enzymes in various fractions is shown in Tables I–III. In respect to all enzymes an increased activity was observed 1–2 days after hepatectomy. The "total" (Triton incubated) specific activity of the homogenate is shown in Fig. 1. Acid phosphatase and β-glucuronidase attained their maximum concentration after 1 day, while the peak for aryl sulfatase was reached after 2 days (Fig. 1). After 3 days all enzymes had attained their initial values, and largely the same values were noted after 10 days.

After 1 day the increase was 29% for acid phosphatase, 87% for β-glucuronidase and 62% for arylsulfatase. The maximum specific activity of aryl sulfatase was 194% of the value for normal liver. With regard to the ratio free/total activity, no obvious variations were observed. There was, perhaps, a tendency towards an increase of this ratio during the first few days of regeneration (Tables I, III), indicating an increase of the free activity.

The recovery ratios, F I/homogenate, were calculated at 5.5 for acid phosphatase, 4.3 for β-glucuronidase, and 5.4 for arylsulfatase.

TABLE I. Specific Activity of Acid Phosphatase (mmoles of P liberated/min/g of protein).

After partial hepatectomy (days)	Hom.	T-Hom.	Free activity (%)	Sup.	T-Sup.	Free activity (%)	F I	T-F I	Free activity (%)
0	4.6 ± 1.1	14.0 ± 0.8	33 (4)	6.5 ± 1.9	10.3 ± 1.5	63 (2)	12.2 ± 0.8	77.1 ± 8.2	16 (4)
1	6.1 ± 1.6	18.1 ± 0.8	34 (4)	5.8 ± 0.3	7.1 ± 0.1	82 (2)	10.0 ± 3.1	53.2 ± 4.2	19 (4)
2	4.1 ± 0.7	17.3 ± 1.9	24 (5)	6.0 ± 1.7	6.4 ± 0.8	94 (5)	9.8 ± 1.6	65.0 ± 3.2	15 (5)
3	3.3 ± 0.4	13.4 ± 1.5	25 (6)	6.0 ± 1.3	10.0 ± 1.2	60 (4)	8.9 ± 0.8	70.1 ± 5.0	13 (6)
10	4.5 ± 0.1	16.9 ± 2.8	28 (3)	4.5 ±	7.7 ±	59 (1)	8.0 ± 0.6	62.2 ± 1.6	13 (3)

Abbrev.: Hom. = homogenate; T-Hom. = total activity (Triton X-100 incubated homogenate); Sup. = supernatant; T-Sup. = total activity (Triton X-100 incubated supernatant); F I = fraction I; and T-F I = total activity (Triton X-100 incubated fraction I). In parentheses are the no. of animals.

TABLE II. Specific Activity of β-Glucuronidase (mg of liberated phenolphthalein/min/mg of protein).

After partial hepatectomy (days)	Hom.	T-Hom.	Free activity (%)	Sup.	T-Sup.	Free activity (%)	F I	T-F I	Free activity (%)
0	34.2 ± 1.4	122.8 ± 8.2	27 (3)	41.6 ± 2.5	73.6 ± 11.5	57 (2)	189.5 ± 16.8	517.4 ± 54.4	37 (3)
1	88.3 ± 11.4	229.3 ± 24.3	27 (3)	157.8 ± 58.3	241.0 ± 48.0	65 (2)	240.8 ± 42.0	544.5 ± 54.0	44 (4)
2	66.2 ± 12.1	147.3 ± 13.8	45 (3)	57.3 ± 10.6	119.3 ± 8.3	48 (3)	132.0 ± 21.0	364.0 ± 18.1	36 (3)
3	38.5 ± 5.0	138.2 ± 13.7	28 (6)	53.1 ± 4.9	106.7 ± 3.8	50 (3)	174.0 ± 19.4	445.0 ± 44.8	39 (6)
10	30.8 ± 7.2	74.5 ± 10.8	41 (3)	39.1 ±	44.5 ±	87 (1)	119.6 ± 13.4	219.0 ± 14.0	54 (2)

See Table I for explanation of abbreviations.

TABLE III. Specific Activity of Arylsulfatase B (mg of liberated nitrocatechol/min/g of protein).

After partial hepatectomy (days)	T-Hom.	T-F I
0	0.89 ± 0.05 (4)	4.78 ± 0.40 (4)
1	1.44 ± 0.49 (4)	4.87 ± 0.69 (4)
2	2.62 ± 0.45 (3)	16.6 ± 1.65 (3)
3	1.03 ± 0.19 (8)	7.23 ± 0.67 (8)
10	0.87 ± 0.13 (6)	7.39 ± 0.98 (6)

See Table I for explanation of abbreviations.

These results correspond to the ratios indicated by Savant *et al.* (18). The free and bound enzymatic activity noted in liver homogenate after sham operation and after manipulation in connection with hepatectomy is shown in Fig. 2. Sham operation had no effect on the activity of the enzymes under study. On the other hand, after manipulation of the liver lobes the free activity of β-glucuronidase increased from 28 to 47%. Acid phosphatase showed no change.

The percentile distribution, after centrifugation, of the increase in specific enzymatic activity between nonprecipitable supernatant and precipitable F I activity is shown in Fig. 3. In order to eliminate the variations in protein concentration the values are expressed as percentages of the total activity. The increase in β-glucuronidase activity after regeneration for 1 day was due mainly to an increase of the nonprecipitable activity. The percentile proportion of F I, on the other hand, showed a much slighter rise during the first few days of regeneration of the liver. The increase in acid phosphatase mainly occurred in the precipitable F I fraction, while the nonprecipitable fraction decreased.

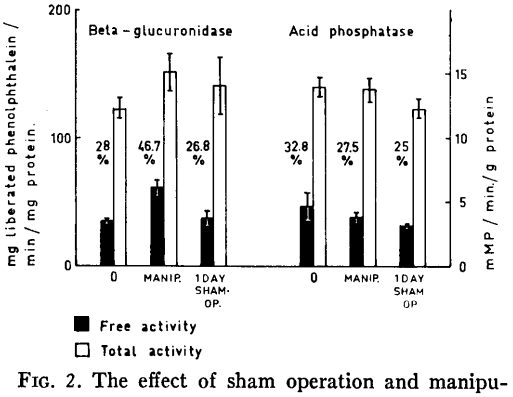

FIG. 2. The effect of sham operation and manipulation of the liver on the free/total enzyme activity in the homogenate.

Discussion. The regenerating liver offers an excellent model for the study of a rapidly growing cell population and for the study of problems relating to the course of cell mitosis. The maximal mitotic activity of the hepatocytes occurs about 24–30 hr after partial hepatectomy, while proliferation of the reticuloendothelial cells and the connective tissue begins 1 day later (1). The maximum DNA synthesis occurs about 6–8 hr before the peak of mitotic frequency (11).

Adams (2) correlated these results with his observations that DNase and acid phosphatase attain their maximal concentration 3–5 hr before the occurrence of DNA synthesis and that the free enzymatic activity increases at the time of mitosis. According to Keir and Aird (13), a similar activation plays a part in certain "priming reactions."

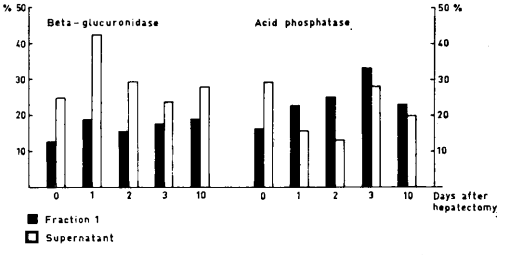

FIG. 3. Yield of total enzyme activity (%) in the supernatant and F 1 fraction.

In the present study, acid phosphatase, β-glucuronidase and arylsulfatase B were determined during a longer period of time than in Adams' (2) investigation. Since the three enzymes investigated showed no simultaneous increase in activity, it may be assumed that as a cellular structure the lysosomal organelle does not initiate mitotic activity. The maximum concentrations are attained too late for these enzymes to be regarded as primary factors in the synthesis of DNA and RNA which occurs within the first 24 hr after hepatectomy. The present results argue in favor of the view that the activation of the lysosomal particles occurs at a later point of

time than the initiation of mitosis. This view is consistent with the results of Becker and Lane (5). These authors assumed that lysosomal enzymes occur within the autophagosomes in connection with the induction of mitotic cell division. On the other hand it is possible that the autolytic enzymes act as inhibitory agents after the initiation of mitotic activity. In addition, it seems possible that the increase in acid phosphatase observed in lymphocytes relates to the transport across the cell membrane (7). With regard to the effect of sham operation on the enzyme concentration in regenerating liver, contradictory views have been published (3, 20). The present results support the view that this effect is inconsiderable. Manipulation in connection with hepatectomy seems to release lysosomal enzymes surrounded by a membrane.

Summary. The concentration of the lysosomal enzymes acid phosphatase, arylsulfatase B and β-glucuronidase, was determined in regenerating liver 1, 2, 3, and 10 days after partial hepatectomy. An obvious increase in enzymatic activity was observed 2–3 days after the operation, but the reactions of the different enzymes were not parallel. It is concluded that the lysosomal organelle as such does not participate in the initiation of the process of cell division. The enzymatic activities seem to be at their maximum at a point of time subsequent to mitotic division. The present results argue in favor of the view that the lysosomes play a part in a postmitotic autophagocytosis. It is suggested that the lysosomal enzymes are involved in the inhibition of the regeneration of the liver.

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