THE MITOTIC RESPONSE IN THE RAT LIVER AFTER DIFFERENT REGENERATIVE STIMULI

K. WEINBREN AND EVELYN TARSH

From the Department of Pathology, Postgraduate Medical School, London W.12

Received for publication January 16, 1964

ALTHOUGH not conclusive, there is evidence that the mechanism by which the liver is restored after partial hepatectomy involves some sort of humoral mediation (Glinos, 1958 ; Smythe and Moore, 1958 ; Stich and Florian, 1958 ; Paschkis, 1958). This problem has proved a difficult one and much of the evidence supporting this idea is circumstantial. Direct inhibition or stimulation of regenerative growth have not been regularly achieved by the use of cell-free fractions of liver tissue or of serum and it may be that optimal conditions for demonstrating these effects have not yet been established.

The standard 2/3 partial hepatectomy in the rat has been used as a model in most of the experiments designed to investigate liver restoration although it seems possible that some other form of damage might stimulate a type of restoration more suitable for analysis of this problem. Many different procedures are known to destroy or alter liver cells and to be associated with hyperplasia of those cells or regions which are not directly affected. Included among these are surgical operations applied to one or more of the vessels supplying part of the liver and Steiner and Martinez (1961) have recently drawn attention to the great difference in microscopical appearance of liver tissue after different vessels are occluded. In an attempt to compare the restoration after different hepatic structures had been ligated, Lawrence, Joly and Brasfield (1959) examined the remnants or " surgically unmanipulated " lobes of the rat liver 4 weeks after the operation. They did not report on any changes in the restorative process during the first few days when mitotic activity is at its height. It is, however, in this early period when the effects of growth-regulating factors are sought.

Another variable which may influence the pattern of the proliferative response is the quantity of parenchyma affected by the damage ; this question has been investigated after excision of different proportions of the liver and the remnant has been examined by several different procedures (Drabkin, 1947 ; MacDonald, Rogers and Pechet, 1962 ; Weinbren and Woodward, 1964).

This paper reports a comparison of the mitotic responses in the posterior lobes of the rat liver to partial hepatectomy, infarction or atrophy due to portal deviation of the anterior two lobes, and also a comparison of the responses when similar procedures are applied to lesser amounts of liver tissue.

MATERIAL AND METHODS

Male white rats bred in our own laboratory were used. The litters were reduced to eight at birth, weaning took place at three weeks and the animals were fed on " Research " rat cubes. They were not fasted before operations or death.

The experimental procedures, always under ether anaesthesia, involved :

1. Partial hepatectomy, removing 2/3 of the liver, by the method of Higgins and Anderson (1931).
2. Infarction of the 2 anterior lobes of the liver, by ligation of portal vein, hepatic artery and bile duct at the hilum of the 2 anterior lobes.
3. The induction of atrophy of the 2 anterior lobes, by ligation of the relevant branch of the portal vein only, leaving the artery and duct intact.
4. Each of the above mentioned procedures applied to the caudate lobe only.
5. Induction of atrophy and infarction to right lobe only.

Rats were killed by ether inhalation, the livers being removed while the animals were still under anaesthetic. The animals were killed in groups of 6 or more after 24, 29, 36, 48, 60 and 72 hr. All operations were performed between 10 a.m. and noon. Experiments were rejected as unsatisfactory if lobes other than the intended lobes showed evidence of vascular damage or if macroscopic necrosis was found in lobes deprived of portal blood or if dye injected into the spleen was found in tissue from which it was intended to deviate the portal blood flow.

Tissues were fixed in 4 per cent formaldehyde, paraffin wax sections cut at 7 μ and stained with haematoxylin and eosin.

Mitoses were counted as described previously (Weinbren, 1955) using a square ocular and the incidence was expressed per 1000 nuclei. Deoxyribonucleic acid (DNA) was estimated by a method based on the procedure of Smellie, Kier and Davidson (1959) in lobes deprived of portal blood supply of 5 rats 3 days after operation and in 5 controls. Extraction with 0·5 N-perchloric acid was repeated 3 times and the final extract was digested with 10 N-$HClO_4$ in Kjeldahl tubes and the phosphate determined by the method of Allen (1940).

RESULTS

The results are summarised in the Tables I, II and III and in the graphs (Fig. 1–3). Of 13 normal rats, the anterior lobes weighed 66·6 $\pm$ 1·4 (S.D.) per cent of the total liver weight the caudate lobe 8·6 $\pm$ 1·4 per cent and the right lobe 24·6 $\pm$ 1·9 per cent. In 12 normal male rats of the same weight as the others, the incidence of mitotic figures in the livers was between 1·7 and 2·2 per 1000 cells. In Group 1 (partial hepatectomy) the mean at 24 hr. was 20 mitoses per 1000 cells, in Group 3 (portal vein ligation) this was 12 and in Group 2 (infarction) 9 mitoses

TABLE I.—*The Incidence of Mitoses after Different Procedures Applied to Anterior Two Lobes of the Rat Liver* (67 *per cent*)

Time (hr.)	Procedure	Number of rats	Mitoses per 1000	±S.E.
24	Resection	5	20·0	6·9
	Infarction	5	9·2	3·6
	Atrophy	6	12·3	9·5
29	Resection	10	37·1	5·9
	Infarction	4	19·2	2·9
	Atrophy	10	47·1	6·9
36	Resection	6	15·7	4·3
	Infarction	5	10·4	4·6
	Atrophy	4	17·1	5·7
48	Resection	6	25·0	1·7
	Infarction	6	23·1	2·7
	Atrophy	6	36·4	7·3
60	Resection	7	7·5	2·4
	Infarction	4	26·0	11·3
	Atrophy	5	7·9	0·7
72	Resection	12	21·2	2·4
	Infarction	7	13·7	4·7
	Atrophy	7	21·6	3·6

TABLE II.—*The Incidence of Mitoses after Different Procedures Applied to Caudate Lobe of the Rat Liver* (8·6 *per cent*)

Time (hr.)	Procedure	Number of rats	Mitoses per 1000	±S.E.
24	Resection	6	0·04	0·18
	Infarction	6	0·01	0·09
	Atrophy	7	0	—
29	Resection	6	0·45	0·19
	Infarction	6	2·00	0·40
	Atrophy	4	0·45	0·10
36	Resection	6	1·16	0·47
	Infarction	5	2·31	0·64
	Atrophy	4	0·80	0·62
48	Resection	7	2·16	0·47
	Infarction	5	1·62	1·43
	Atrophy	4	0·50	0·01
60	Resection	6	1·10	0·37
	Infarction	5	0·94	0·26
	Atrophy	5	0·74	0·26
72	Resection	6	4·90	0·88
	Infarction	5	3·50	1·90
	Atrophy	6	5·60	1·69

TABLE III.—*The Incidence of Mitoses after Different Procedures Applied to Right Lobe of the Rat Liver* (24·6 *per cent*)

Time (hr.)	Procedure	Number of rats	Mitoses per 1000	±S.E.
24	Infarction	6	4·1	3·4
	Atrophy	9	3·8	1·5
29	Infarction	7	3·7	1·6
	Atrophy	6	13·5	2·0
48	Infarction	6	6·6	2·9
	Atrophy	13	14·3	2·7
72	Infarction	5	4·9	3·8
	Atrophy	6	6·3	1·6

per 1000 cells. At 29 hr. Groups 1 and 3 showed an increase to 37 and 47 respectively, but this was not so marked in Group 2 (19/1000). Thereafter a distinct diurnal rhythm in the incidence of mitoses was observed in Groups 1 and 3, lower levels being found near midnight and higher levels near midday.

Groups 1 and 3 achieved the highest level in the first 29 hr. whereas the rats in Group 2 showed a higher level between 48–60 hr.

There was no significant rise in the incidence of mitoses until 72 hr. in those animals in which the surgical procedure was applied to only the caudate lobe of the liver (Group 4). A raised level of mitoses was found at all times examined when infarction or atrophy were induced in the right lobe (Group 5). The levels were roughly proportional to the amount of tissue affected in comparison with similar procedures applied to the anterior lobes. Resection is not possible in the right lobe because of its relation to the inferior vena cava.

The DNA content of the atrophied lobes in Group 3 is shown in Table IV and, compared with normal liver there appears to be no significant reduction in total DNA in the lobes deprived of portal blood flow. The microscopical appearance of these lobes confirmed previous descriptions, only very small necrotic foci being found.

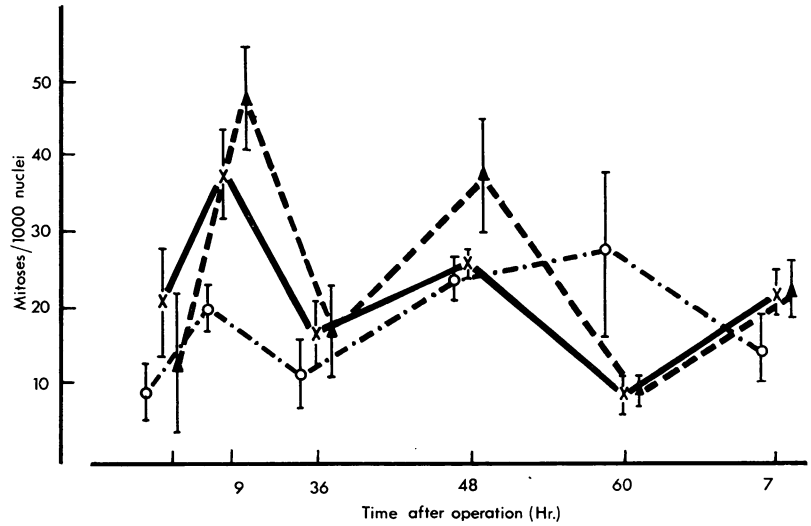

FIG. 1.—Incidence of mitoses in " unmanipulated " right lobe after different stimuli to anterior lobes (67 per cent of liver).

———— × ———— Resection of anterior lobes.
—— . ——○—— . —— Infarction of anterior lobes.
– – – – – ▲ – – – – – Atrophy of anterior lobes.

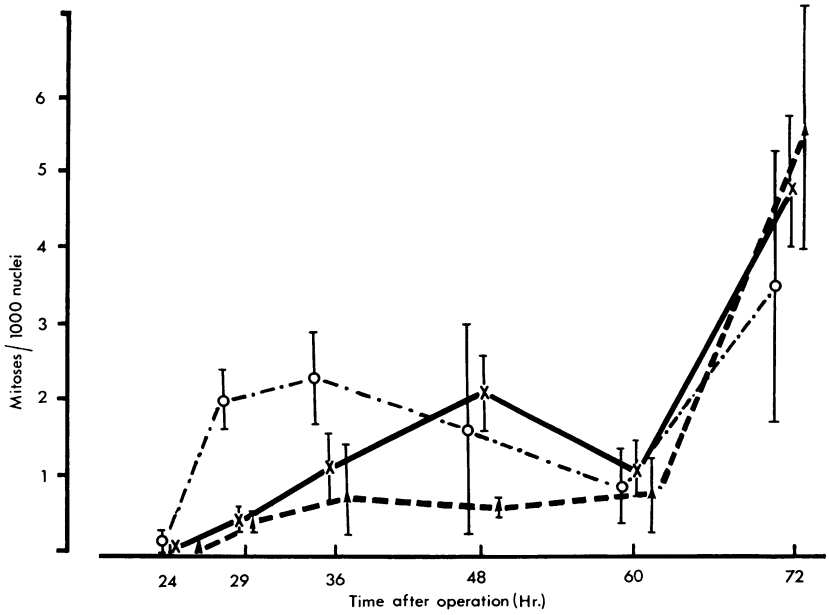

FIG. 2.—Incidence of mitoses in " unmanipulated " right lobe after different stimuli to caudate lobe (8·6 per cent of liver).

———— × ———— Resection of caudate lobe.
—— . ——○—— . —— Infarction of caudate lobe.
– – – – – ▲ – – – – – Atrophy of caudate lobe.

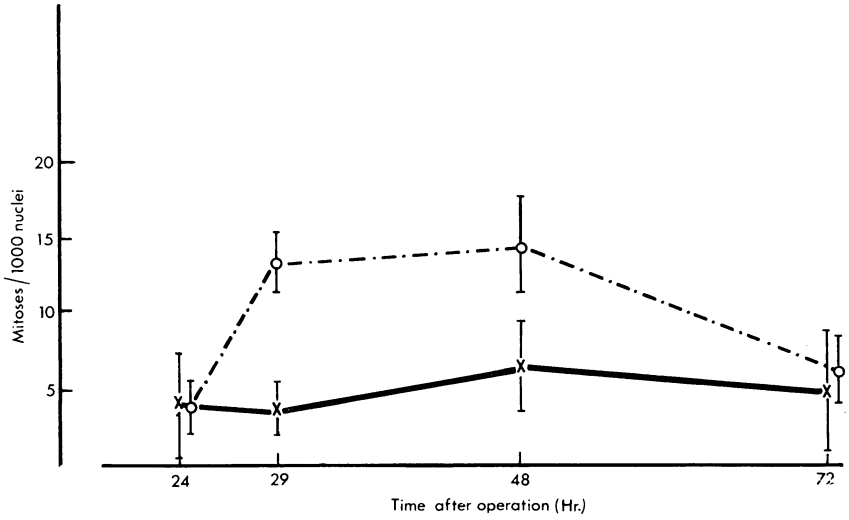

FIG. 3.—Incidence of mitoses in "unmanipulated" caudate lobe after different stimuli to right lobe.

——·——○——·—— Atrophy of right lobe.
——————×—————— Infarction of right lobe.

The vertical lines represent ±S.E. of mean.

TABLE IV.—*The Deoxyribonucleic Acid Content of Liver Tissue Three Days after Deprivation of Portal Blood Flow*

Experiment	DNA P (μg.) in 300 mg. liver
1	89·7
2	85·1
3	131
4	78
5	131
Control	
6	65
7	70
8	60·2
9	57
10	60

DISCUSSION

The results confirm the diurnal periodicity of the incidence of mitoses after partial hepatectomy (Jaffee, 1954), and indicate that a similar rhythm occurs in the "unmanipulated lobes" after deviation of the portal flow. The level of mitoses in the posterior lobes is as great when the portal blood flow is deviated from the two anterior lobes as when a 2/3 partial hepatectomy is performed. Lawrence *et al.* (1959) found that after four weeks there was a difference in weight between the posterior lobes after removal of the anterior lobes and the corresponding lobes after deviation of the portal blood from the anterior lobes. They concluded that this might be interpreted as less restoration in the latter case. The present findings cannot be reconciled with this interpretation and it appears to be more reasonable to consider that the final weight of liver tissue in long-term (4 weeks) experiments may be the result of different factors. It has previously been shown (Weinbren, 1955; Duchen, 1961) that although there is a rapid atrophy

of liver tissue deprived of portal blood flow, this continues for only 14–18 days after which there is no further atrophy. From this time on, this lobe retains its reduced weight and the posterior lobes no longer increase in size. The equilibrium that is maintained at this time may involve a mechanism quite different from that which initiated hepatic restoration during the first few days of intense mitotic activity (Weinbren, 1959). The retained DNA level and absence of destruction in the tissue deprived of portal blood, both suggest that the stimulus for regeneration does not depend on destruction or removal of nuclear material. It may be reasonable to postulate that a cytoplasmic component is involved in the production of a possible chemical mediator in this process, as the observed atrophy involves the cytoplasm almost entirely.

Why the infarcted anterior lobes should stimulate a slightly different response from that induced by the other procedures is not clear, but the rather wider scatter of mitoses may imply a slower development of the lesion related perhaps to less certain technical procedures, such as the exact level of the placing of the ligature.

The late development (72 hr.) of a mitotic response in the caudate lobe procedures was a constant finding in all these groups. This may indicate the need for examining the tissues at various times in view of the fact that the liver tissue undergoing restorative hyperplasia may show a variation in incidence and also in time of development of mitotic figures.

SUMMARY

Mitotic responses in the posterior lobes of the rat liver were compared after removal atrophy or infarction of the anterior lobes. In other series these different stimuli were applied to smaller portions of liver tissue.

The incidence of mitoses did not vary much with the different stimuli, and a striking finding was the early initiation and high level of mitotic figures in posterior lobes after atrophy was induced in the anterior lobes.

We are grateful to the Medical Research Council for a grant supporting this work, and the London Hospital for supplying apparatus which we used for much of this work.

REFERENCES

ALLEN, R. L. J.—(1940) *Biochem. J.*, **34**, 858.
DRABKIN, D. L.—(1947) *J. biol. Chem.* **171**, 395.
DUCHEN, L. W.—(1961) *Brit. J. exp. Path.*, **42**, 247.
GLINOS, A. D.—(1958) in ' Liver Function ', ed. R. W. Brauer. Washington D.C. (Am. Inst. of Biological Sciences), p. 433.
HIGGINS, E. M. AND ANDERSON, R. M.—(1931) *Arch. Path.*, **12**, 186.
JAFFEE, J. J.—(1954) *Anat. Rec.*, **120**, 935.
LAWRENCE, W., JOLY, D. AND BRASFIELD, R.—(1959) *Surgery*, **45**, 543.
MACDONALD, R. A., ROGERS, A. E. AND PECHET, G.—(1962) *Lab. Invest.*, **11**, 544.
PASCHKIS, K. E.—(1958) *Cancer Res.*, **18**, 981.
SMELLIE, R. M. S., KIER, H. M. AND DAVIDSON, J. N.—(1959) *Biochim. biophys. Acta.*, **35**, 389.
SMYTHE, R. L. AND MOORE, R. O.—(1958) *Surgery*, **44**, 561.
STEINER, P. E. AND MARTINEZ, J. B.—(1961) *Amer. J. Path.*, **39**, 257.
STICH, H. F. AND FLORIAN, M. L.—(1958) *Canad. J. Biochem. and Physiol.*, **36**, 855.
WEINBREN, K.—(1955) *Brit. J. exp. Path.*, **36**, 583.—(1959) *Gastroenterology*, **37**, 657.
Idem AND WOODWARD, ELSA—(1964) *Brit. J. exp. Path.*, **45**, 442.