Brit. J. Pharmacol. (1949), **4**, 93.

THE EFFECT OF HEPATECTOMY ON THE ACTION OF CERTAIN ANAESTHETICS IN RATS

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(Received September 30, 1948)

To investigate the role of the liver in the detoxication of anaesthetics, two main methods are available. First, the liver may be damaged by some drug such as carbon tetrachloride; if the anaesthetic has a greater effect than before, it is probable that it is normally detoxicated by the liver. This method is open to at least two objections. In the first place, it is impossible accurately to assess the amount of liver damaged by this means; and further it is possible that the carbon tetrachloride has some action of its own on the central nervous system. The second method, which we have used, is to remove by operation a large proportion of the liver and observe whether the effect of the anaesthetic is increased. This method has the advantage that the amount of liver tissue removed can be estimated fairly accurately, and it is unlikely that the operation has any complicating side-effects. Higgins and Anderson (1931) described a method for partial hepatectomy in rats, and the high survival rate and quick recovery of the animals make it suitable for this type of investigation.

METHOD

Albino rats weighing between 150 and 300 g. were used. Each rat was given a preliminary dose of the drug to be tested in the following manner. Food was withheld overnight, and the following morning the drug was given to the animal by stomach-tube in a dose calculated according to body-weight. At frequent intervals the rat was laid on its side, and when it remained in this position it was considered to be asleep. As soon as it got to its feet it was considered awake, and the sleeping-time was thus determined. At least five days later partial hepatectomy was carried out by the method of Higgins and Anderson (1931). The animals withstood the operation well, the mortality being about 10 per cent, and by the third day after operation appeared perfectly normal. For 24 hours after operation they were fed on bread and milk and 20 per cent glucose. Thereafter they were given the normal laboratory diet. On a chosen day after hepatectomy, the rat was given the same dose of drug as before, and the sleeping-time again determined in the same way. The experiment was planned to give at least 10 results on each of the following days after hepatectomy: 6th–7th, 8th–9th, 10th, 12th, 15th, 17th–18th, 20th, 22nd, and 26th–28th. The mean sleeping-time of the rats on each of these days after

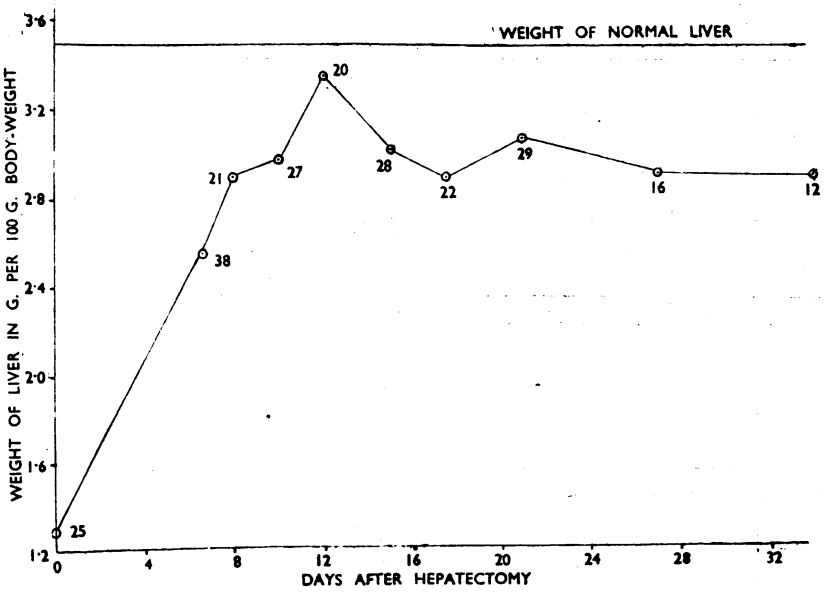

FIG. 1.—Graph showing regeneration of liver by weight after hepatectomy. Numbers at each point show the number of rats used to determine each mean.

operation could then be compared with their mean sleeping-time before operation.

Immediately each experiment was finished the rat was killed and its liver removed, washed in saline, dried with filter-paper, and weighed. No rat was used twice after hepatectomy.

The drugs and doses used were: chloral hydrate, 350 mg. per kg.; bromethol, 300 mg. per kg.; soluble phenobarbitone, 110 mg. per kg.; thiopentone, 50 mg. per kg.

RESULTS

1. *Rate of regeneration of liver*

In order to obtain an average figure for the amount of liver removed at operation a preliminary experiment was carried out on 40 rats. The animals were hepatectomized and the portion of liver removed was weighed. The animals were then killed and the remainder of the liver was dissected out and weighed. From these two weights, the mean percentage of liver removed was calculated, and found to be 62.2 per cent by weight (S.D. = 4.88 per cent). This figure is lower than that of 70 per cent found by Higgins and Anderson (1931). Expressed as g. per 100 g. body-weight of rat, the mean weight of liver left behind after operation in these 40 rats was 1.29 (S.D. = 0.28) and the mean weight of the whole liver was 3.49 (S.D. = 0.81).

TABLE I

DIFFERENCES IN MEAN SLEEPING-TIME OF GROUPS OF RATS ON VARIOUS DAYS AFTER HEPATECTOMY. DEATHS SHOWN IN COLUMN (IV) ARE INCLUDED IN THE FIGURES IN COLUMN (V) EACH AS 500 MIN. SLEEPING-TIME

(i)	(ii)	(iii)	(iv)	(v)	(vi)
Number of rats	Day after hepatectomy	Mean sleeping-time in min. before hepatectomy	Number of deaths due to effect of drug after hepatectomy	Mean sleeping-time in min. after hepatectomy	Difference (v)–(iii)
CHLORAL HYDRATE (350 mg. per kg.)					
16	6–7	171.1	4	346.1	+175.0
10	8–9	162.0	6	373.7	+211.7
10	10	85.2	4	277.4	+192.2
10	12	85.7	3	341.0	+255.3
10	15	209.4	7	384.0	+174.6
10	17–18	78.0	1	112.9	+34.9
10	22	176.3	0	74.8	−101.5
6	28	147.0	0	97.5	−49.5
BROMETHOL (300 mg. per kg.)					
16	6–7	59.7	8	416.6	+356.9
11	8	91.0	8	413.0	+322.0
10	10	77.5	6	396.6	+319.1
10	12	24.1	7	375.5	+351.4
10	15	111.7	4	281.6	+169.9
10	17–18	98.0	3	265.0	+167.0
10	22	76.7	0	93.5	+16.8
10	26	172.8	0	55.4	−117.4
SOLUBLE PHENOBARBITONE (110 mg. per kg.)					
17	7–8	6.8	1	98.5	+91.7
10	10	0	0	78.1	+78.1
11	12	19.0	0	75.0	+56.0
10	16	0	0	20.5	+20.5
8	28	0	0	0	0
THIOPENTONE (50 mg. per kg.)					
3	4	133.3	2	573.3	+440.0
10	8	94.1	3	314.0	+219.9
8	12	12.0	2	227.0	+215.0
10	15	86.8	1	212.4	+125.6
8	20	92.0	1	110.0	+18.0

Fig. 1 shows the mean weights of the liver (in g. per 100 g. body-weight) of groups of rats on various days after hepatectomy. It will be seen that regeneration proceeded very rapidly during the first week after operation and that the liver had doubled its weight by the 7th day. In our experiments the liver had not regained its original weight by the 34th day, whereas in Higgins and Anderson's experiments it had regained its weight by the 14th day.

Brues, Drury, and Brues (1936) found that there was a fairly constant relationship between the weights of liver removed and that left behind, and therefore expressed the degree of liver regeneration as a percentage of the original liver weight. We have found that this method gives figures with a large scatter and that more consistent results are obtained by using the actual weight of liver in the rat in g. per 100 g. body-weight of rat.

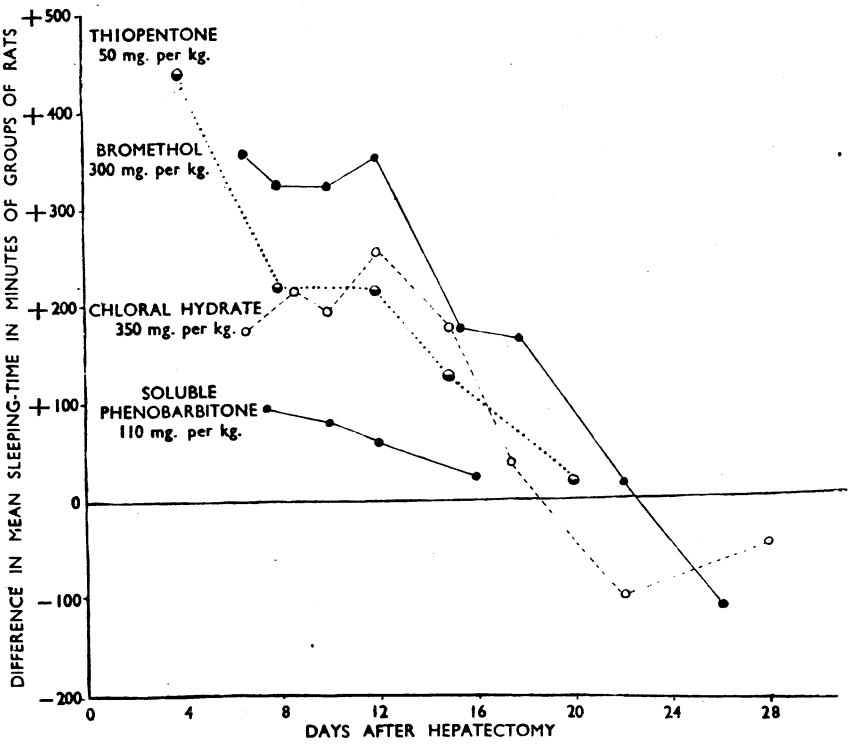

FIG. 2.—Graph showing differences in mean sleeping-times of groups of rats on various days after hepatectomy.

2. *Effect of anaesthetics after hepatectomy*

Table I shows the mean sleeping-times of groups of rats before and on various days after operation. Column (vi) gives the difference between these two figures and shows whether the effect of the drug was increased or not. In the early days after operation the greatly increased effect of the drug resulted in the death of some animals. In order to include these results in the calculations it was necessary to consider them as if they had slept for a long period. Since rats rarely slept after any of the drugs for more than 400 min., death was considered as equivalent to the arbitrary figure of 500 min. sleeping-time.

It can be seen from Table I that up to the 12th day after operation the effect of chloral hydrate was greatly increased. On the 18th day the effect was only slightly greater than that before operation, and on the 22nd day it was actually less. Similarly, Table I shows that by the 22nd day the effect of bromethol was almost the same as before, and was less than before operation on the 26th day. The results for soluble phenobarbitone show that there was a definite though smaller increase in sleeping-time after operation, and that this increase had almost disappeared by the 16th day. It will be seen that, with the dose of phenobarbitone used, animals rarely slept before operation. It was not possible to use a larger dose, which would produce sleep in most animals before operation, without causing a large number of deaths. Table I shows that by the 20th day the effect of thiopentone was almost the same as before hepatectomy. These results are expressed graphically in Fig. 2.

In order to make sure that the increased effect of these drugs after hepatectomy was due to the loss of liver substance and not to the operative interference or anaesthetic, control experiments were done for all four substances. A group of rats was given the usual dose of the drug; five days later laparotomy was carried out, and on the fourth day after operation a second dose of the

TABLE II

TABLE SHOWING THAT THERE IS NO CORRELATION BETWEEN DEGREE OF LIVER REGENERATION BY WEIGHT AND DIFFERENCE IN SLEEPING-TIME AFTER OPERATION. FIGURES ARE PERCENTAGES OF ANIMALS IN EACH GROUP

Drug	(i) Difference in sleeping-time in min.	(ii) Percentages of animals grouped in terms of weight of liver in g. per 100 g. body-weight <2.5g.	(iii) 2.5–3.0g.	(iv) 3.0–3.5g.	(v) >3.5g.
Chloral hydrate 350 mg./kg.	No increase	30	50	36	37
	+1–200	25	18	36	18
	+201–400	40	16	18	27
	+>400	5	16	10	18
Bromethol 300 mg./kg.	No increase	17	47	22	16
	+1–200	28	17	34	12
	+201–400	38	23	22	36
	+>400	17	13	22	36

drug was given as before. It was found that the increase in the mean sleeping-time was negligible.

We observed that rats before hepatectomy never slept for longer than 200 min. with any of the drugs. If the drug had a greater effect the animal died. After hepatectomy, however, the drug often caused rats to sleep for much longer periods. For instance, of 144 normal rats receiving bromethol, the highest sleeping-time was 190 min. Of 69 hepatectomized rats receiving bromethol, 10 slept for longer than 350 min. without dying. This observation is consistent with the idea that after hepatectomy the detoxication processes are slowed.

3. *Correlation of effect of drug with degree of liver regeneration*

We were interested to see if there were any correlation between the degree of regeneration of the liver and its ability to detoxicate anaesthetics. If there were a correlation, we should have expected animals with marked liver regeneration to be less affected by the anaesthetic than those whose livers had only regained a small proportion of their former weight. In Table II animals are arranged in four groups according to the weights of their livers at death (columns (ii), (iii), (iv), and (v)). Each of these groups is divided into four classes according to the difference in sleeping-time after hepatectomy. The figures in each column are the percentages of rats in each group. If there were a correlation, we should expect that a high percentage of animals with small liver-weights would have a much increased sleeping-time after operation, and conversely a high percentage of those with large liver-weights would have little or no increase in sleeping-time. It is clear from Table II that there is no such trend. For instance, for chloral hydrate, 45 per cent of rats whose liver weighed less than 2.5 g. per 100 g. showed an increased sleeping-time after operation of more than 200 min., whereas the same percentage (45) of animals whose livers weighed more than 3.5 g. also had an increase of more than 200 min. sleeping-time. Similarly, for bromethol, 55 per cent of animals with less than 2.5 g. liver per 100 g. and 72 per cent of animals with more than 3.5 g. per 100 g. showed an increase of sleeping-time after operation of more than 200 min. Hence the degree of regeneration of liver by weight is no guide to its ability to detoxicate these drugs.

Discussion

Chloral hydrate and bromethol are usually said to be detoxicated in the liver. Thus, Mukerji and Ghose (1940) found that, in dogs with livers damaged by carbon tetrachloride, there was a well-marked excretion of free chloral in the urine after oral administration of the drug, whereas in normal dogs all the urinary chloral was conjugated. There is, however, little definite evidence that bromethol is detoxicated in the liver, though it has been claimed that it can cause transient liver damage (Bourne and Raginsky, 1931; Coleman, 1938). Our experiments show that both these drugs are detoxicated in the liver, though the possibility that partial hepatectomy affects the renal excretion of them has yet to be excluded. Table II shows that there is no correlation between the degree of liver regeneration by weight and its ability to detoxicate the drug. It is clear that the restoration of the enzyme systems which break down or conjugate these drugs is independent of the restoration of the mass of liver tissue.

Most observers state that phenobarbitone is not detoxicated in the liver. Cameron and De Saram (1939) found that there was no significant prolongation of its action in rats after acute liver damage caused by carbon tetrachloride. Masson and Beland (1945) stated that the anaesthetic effect of phenobarbitone was not increased by partial hepatectomy, whereas after bilateral nephrectomy rats slept for over twice as long as the controls. Similarly, Hirschfelder and Haury (1933) found that bilateral nephrectomy markedly increased the effect of phenobarbitone in rabbits. These and other workers, therefore, consider that soluble phenobarbitone is eliminated through the kidney and not detoxicated to a significant extent by the liver. It is, however, not possible to recover more

than 25 per cent of administered phenobarbitone in human urine (Halberkann and Reiche, 1927), so that 75 per cent is still to be accounted for. Our experiments show that partial hepatectomy increases the effect of soluble phenobarbitone up to the 16th day after operation. The possibility that hepatectomy affects the renal excretion of the drug in some way has yet to be excluded, but a probable explanation is that the drug is detoxicated to some extent in the liver.

It will be seen from Table I that the increase in mean sleeping-time with phenobarbitone on the seventh day after operation (90 min.) is much less than that with chloral and bromethol. This is possibly due to the relatively smaller dose of phenobarbitone used.

The evidence about thiopentone is conflicting. Richards and Appel (1941) found that its effect in rats was not increased after liver damage by carbon tetrachloride, and Scheifley (1946) found that its effect was not increased by partial hepatectomy. On the other hand, Schideman, Kelly, and Adams (1947) found that partial hepatectomy, liver damage with carbon tetrachloride, and the production of an Eck fistula all markedly increased the effect of thiopentone. They also showed that the drug was broken down when incubated *in vitro* with liver slices. Our results support these authors' conclusions that thiopentone is detoxicated in the liver.

Fig. 2 shows that between the 22nd and 26th day the liver appears to be better able to detoxicate chloral hydrate and bromethol than before hepatectomy. Further work is necessary to confirm and elucidate this phenomenon. It is impossible to say whether the same holds good for phenobarbitone, since, with the dose used, animals seldom sleep before operation.

Summary

1. A method is described of determining whether anaesthetics are detoxicated in the liver, by using partial hepatectomy in rats.

2. The anaesthetic effect of chloral hydrate, bromethol, soluble phenobarbitone, and thiopentone is increased by partial hepatectomy. This increased effect is no longer present for chloral hydrate by the 17th, for bromethol by the 22nd, for soluble phenobarbitone by the 16th, and for thiopentone by the 20th, day after operation.

3. There is no apparent correlation between the regeneration of the liver by weight and its power to detoxicate these drugs.

We should like to express our thanks to Professor J. H. Burn for suggesting this work. One of us (J. M. W.) was maintained by a personal grant from the Medical Research Council.

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