A TECHNIQUE FOR TOTAL HEPATECTOMY IN THE RAT AND ITS EFFECT ON TOXICITY OF OCTAMETHYL PYROPHOSPHORAMIDE.

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OCTAMETHYL pyrophosphoramide (OMPA) is the active principle in a number of insecticides that are being used on an increasingly wide scale. An account of some of its toxic properties has been given by Dubois, Doull and Coon (1950). It has also been used successfully for the treatment of myasthenia gravis (Rider, Schulman, Richter, Moeller and Dubois, 1951).

When a toxic dose of OMPA is administered to an animal the signs of poisoning by a cholinergic drug make their appearance within 15–30 minutes and the level of cholinesterase in the blood and some of the tissues falls. For rats, an average lethal dose of OMPA is 8 mg. per kg., and is the same if the drug is administered orally or by parenteral injection.

On the other hand, OMPA, unlike other cholinergic poisons, has no inhibitory action on blood or tissue cholinesterase *in vitro*. However, when liver slices or liver mince is incubated with OMPA an active inhibitor of cholinesterase is produced. No other tissues examined could be shown to possess this power of converting OMPA *in vitro* to an active inhibitor of cholinesterase (Dubois *et al.*, 1950 ; Gardner and Kilby, 1950 ; Aldridge, 1951).

It was, therefore, of interest to find out whether the conversion of OMPA into a cholinesterase inhibitor takes place in the liver of an intact animal.

For this purpose we devised a method by which the rat, a convenient small laboratory animal, could be rendered liverless. The main problem to overcome was that of establishing an adequate collateral circulation for the portal vein and the inferior vena cava before the liver is removed. After considerable trial this difficulty was overcome and a successful 2-stage technique for total hepatectomy in rats was developed.

The hepatectomized rats survived as long as 10 hours without any treatment. The technique will be described in some detail, because it may be of value to those wishing to use rats for metabolic experiments. While this paper was being prepared a brief reference to a similar technique in rats was found (Byers, Friedman and Michaelis, 1951), but no operative details were given.

The effect of injecting OMPA into the hepatectomized rats was observed. The animals survived the administration of doses that killed control animals in 12–23 minutes.

TOTAL HEPATECTOMY

First stage.

Large rats weighing over 300 g. are used. Under ether anaesthesia and observing simple aseptic precautions a right paramedian incision 6 cm. long is

made from the costal margin. After placing a pad of cotton-wool 1–2 cm. thick under the animal's spine at the level of the last rib, intestine is delivered outside the wound, wrapped in a moist towel and drawn to the left. This exposes the right lobe of the liver and the inferior vena cava. The ligament connecting the inferior pole of the right posterior lobe of the liver to the inferior vena cava is divided and a segment of inferior vena cava above the entrance of right renal vein is dissected free in its bed of tissue. Polythene (" alkathene," gauge 300, I.C.I. product) and cellophane films of roughly 15 mm. square are sterilized by boiling in water and placed together. The two wet films adhere closely together and can be treated as a single sheet. A band of about 3 mm. in width is cut from this and passed round the isolated segment of the inferior vena cava with cellophane as the inner layer. After placing a tapering glass rod longitudinally on the vein, the two ends of the constricting band are tied together with a single stitch of fine steel wire so as to include both the rod and the vein. When the glass rod is removed, the inferior vena cava is left half occluded by the band. Further vascular constriction is produced by the fibrosis resulting from the presence of the cellophane. The redundant ends of the band are cut off. The median and left lateral lobes of the liver are gently retracted upwards and the portal vein is exposed. Near its entrance into the liver it is dissected free from adjacent soft tissue, the hepatic artery and the common bile duct. A constricting band is also placed round it to occlude half of its lumen. The incision is closed in two layers with fine steel wire. No special post-operative care is required. At least 6 weeks should be allowed for the development of adequate collateral circulation.

Second stage.

The rat is deprived of food for 16 hours. Under ether anaesthesia the abdomen is opened in the midline starting on the xiphisternum and continuing for about 7 cm. posteriorly. The dilated abdominal veins should not be injured. A pad of cotton-wool is placed under the spine to raise the upper part of the abdominal wall. The median and the left lateral lobes of the liver are expressed outside the incision from the cupola of the diaphragm by squeezing the lateral sides of the upper abdominal wall with the fingers. After dividing the falciform ligament down to the vena cava, the left coronary ligament is exposed by drawing down the left lateral lobe of the liver and divided. The two lobes are cut off after their pedicle has been securely ligated with linen thread. The gastro-hepatic omentum is split longitudinally in the middle and the anterior portion of the caudate lobe is delivered outside the opening. The posterior portion of the caudate lobe, which is situated behind the oesophagus, is also brought outside through the same opening after dividing the overlying peritoneal fold which is partly attached to its ventral surface. The medial side of the remaining liver is now free. Turning to its lateral side, the right coronary ligament is cut after exposing it by holding the right lobe of the liver and pulling it downwards. The right lobe is lifted from the posterior abdominal wall to expose the peritoneal fold between the liver and the crus of the diaphragm. It is divided, and two linen threads are passed from the lateral side of the liver through the opening to its medial side. The two ends of one thread are tied in a loose half hitch round the inferior vena cava between liver and diaphragm.

The first operation invariably causes formation of adhesions between the liver, duodenum and omentum and around the constricting bands. However, if the operation for the first stage has been skilfully done with a minimum of trauma the adhesions are not great, enabling these parts to be separated by rubbing off the liver with gauze and the fingers while controlling the oozing of blood during their separation by compression. When this is done, the two ends of the second thread are brought to the front under the liver and tied tightly. This ligature embraces the portal vein, the bile duct and the inferior vena cava below the liver. The first ligature is then tightened and the intervening liver is cut off as close to the ligature as possible. After removing the spilled blood from the peritoneal cavity, a gauze sponge is left in the operated site to facilitate haemostasis. The abdominal wound is closed with two layers of linen sutures. The operation lasts about 15 minutes. However, if adhesions are extensive much time is consumed in separating them, with great loss of blood. These animals are in very poor general condition after the operation and die soon afterwards. Thus such animals are best discarded.

Clinical Course.

On recovering from the anaesthetic the rat sits up and remains quiet most of the time, but occasionally moves about. It appears anxious and irritable. Later, marked weakness is apparent with sprawling limbs and head resting on the floor. When moribund it lies on its side. The respiration also becomes deeper and slower and Cheyne-Stokes breathing appears in the terminal stage. Fine fibrillary muscular tremors, starting from the head and becoming generalized later, precede the convulsions, which are usually associated with marked opisthotonos. Occasionally the animal rolls on its side. It dies after 2 or 3 such convulsions with respiration stopping before the heart beat.

In our series the hepatectomized rats lived 3–10 hours without administration of glucose. Their period of survival after operation depends on technical skill, extent of operative trauma, amount of blood loss, adequacy of collateral circulation and on keeping the rat quiet without disturbance.

Effect of OMPA on Hepatectomized Rats.

A 1 per cent. aqueous solution of OMPA in a dose of 30 mg. per kg. body-weight was injected into the external jugular vein of male rats immediately after total hepatectomy and after sham operation. A third group of rats was only given an intravenous injection of 0·9 per cent. saline after total hepatectomy.

The results show that liverless rats are able to survive a dose of OMPA sufficient to kill sham-operated rats in 12–23 minutes (Table 1). The mean survival time

TABLE I.—*Period of Survival of Hepatectomized and Sham-Operated Rats after Intravenous Injections of OMPA (30 mg. per kg. body-weight).*

Group.	No. of rats	Period of survival. Mean ± S.E.	Range.
Sham-operated, OMPA injected.	12	17·50 ± 0·89 mins.	12–23 mins.
Hepatectomized :			
a. OMPA injected.	11	5·25 ± 0·33 hrs.	3·2–7·1 hrs.
b. Saline injected.	11	6·05 ± 0·78 hrs.	3·0–10·9 hrs.

of the hepatectomized rats given OMPA was not significantly shorter than that of hepatectomized rats injected with saline only. Both groups of rats died with hypoglycaemic convulsions, but those given OMPA showed some signs of poisoning, namely lachrymation, salivation and muscular twitchings, which developed 4–5 hours after injection. This suggests that some tissue or tissues other than the liver could bring about a slow conversion of OMPA to an active anticholinesterase agent.

DISCUSSION.

These experiments have shown that it is possible to perform total hepatectomy on rats, their survival periods being up to 10 hours. Earlier experiments showed that success depends to a great extent upon the development of an adequate collateral circulation, and a minimum period of 6 weeks seems to be necessary for this. Such rats prove to be just as sensitive to the action of an active anticholinesterase drug, e.g., *p*-nitrophenyl diethyl phosphate (E. 600), as normal or sham-operated animals.

Nothing is known about the chemical reactions involved in the conversions of OMPA into an active cholinesterase inhibitor, nor has the inhibitor been identified or even suggested. The present experiments indicate that the change only takes place at an effective rate in the liver, and they confirm the results of *in vitro* experiments.

SUMMARY.

1. A 2-stage technique for total hepatectomy in rats is described.

2. Hepatectomized rats survived the injection of a dose of OMPA which killed sham-operated rats.

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