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EFFECTS OF VARIOUS ANAESTHETICS ON THE METABOLISM AND GENERAL CONDITION OF THE ISOLATED PERFUSED RAT LIVER

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Abstract—Various anaesthetics have been added to the perfusion medium of an isolated perfused liver preparation and their effect on glucose and urea production were measured. The general condition of the liver was also assesed using the criteria of liver potassium levels, water content and bile secretion.

Pentobarbitone sodium (Nembutal), thiopentone sodium (Pentothal) and tribromoethanol (Avertin) all caused a marked inhibition of glucose output, urea production and bile secretion from the liver. Halothane (Fluothane) had relatively little effect on glucose and urea production at a perfusate concentration fifteen to twenty times that present in the blood of rats under deep halothane anaesthesia, and the effect was immediately reversed after administration of the vapour was stopped. The criteria adopted also showed that it caused little or no structural damage at the same concentration. Trichloroethylene (Trilene), at a perfusate concentration of about five times that to be expected in the blood of an anaesthetized rat, inhibited glucose and urea output, and the loss of intracellular potassium and gain of water indicated that it caused extensive damage to the liver. It is therefore likely to be potentially damaging in man.

The liver potassium levels of rats kept under trichloroethylene anaesthesia for 30 min were slightly but significantly lower than those of rats under halothane anaesthesia for the same length of time.

The production of liver necrosis by halogenated hydrocarbons is discussed in the light of these results.

THIS paper is based on preliminary experiments carried out during the development of an isolated perfused liver system to be used for a study of amino acid metabolism. The experiments were designed to find the most suitable anaesthetic for the removal of the liver from the donor rat, but the results are of more general interest because they provide information about the biochemical effect of various anaesthetics on the liver and about the nature of liver damage.

MATERIALS AND METHODS

Liver perfusions

The isolated perfused rat liver preparation used was basically that of Miller[1] as set up in this department by Fisher and Kerly[2] but modified for the use of Krebs–Ringer bicarbonate buffer[3] as perfusion medium instead of rat blood. Full details of the perfusion technique and operation procedure will be published elsewhere.[4]

Administration of anaesthetics to animals

(*a*) *Injected anaesthetics.* Pentobarbitone sodium (veterinary Nembutal, Abbott Laboratories), thiopentone sodium (Pentothal, May and Baker) and tribromoethanol

(Avertin, Bayer) were given by intraperitoneal injection, the two latter as freshly prepared 2·5-g/100 ml aqueous solutions. The doses per 100 g body weight were 0·08 ml (equivalent to 4·8 mg), 0·3 ml and 0·8 ml respectively.

Methohexitone sodium (Brietal sodium, Eli Lilley) was not used because it was found not to be absorbed by the intraperitoneal route.

(*b*) *Inhalation anaesthetics.* Halothane (2-bromo-2-chloro-1:1:1-trifluoroethane, Fluothane, I.C.I.) and trichloroethylene (Trilene, I.C.I.) were administered as follows: a rat was placed, in its cage, inside a transparent plastic anaesthetizing chamber, and left to settle down for 20–30 min. A mixture of the anaesthetic vapour and oxygen, obtained by bubbling oxygen at a suitable rate through the liquid anaesthetic in a bottle immersed in a water bath at 20° for halothane and at 50° for trichloroethylene, was passed into the chamber to induce anaesthesia. The rat was removed and anaesthesia maintained during the operation by passing oxygen into a 100 ml beaker containing cotton wool moistened with the anaesthetic and held in position over the rat's head. The required depth of anaesthesia and adequate respiration were maintained by adjustment of the position of the beaker and the oxygen flow rate. All anaesthetics were given without any other medication.

Administration of anaesthetics to the perfused liver

(*a*) *Injected anaesthetics.* Available evidence[5–9] suggests that for the injected anaesthetics in question, most of the anaesthetic administered *in vivo*, especially intraperitoneally or parenterally, is taken up by the liver. It therefore seemed reasonable to add to the perfusion reservoir an amount equal to that required to anaesthetize the donor rat. This amount may be as much as twice that taken up by the liver *in vivo* but, owing to the greater volume, its concentration in the perfusate will be approximately only one fifth the maximum blood level.

(*b*) *Inhalation anaesthetics.* Halothane and trichloroethylene could not be added directly to the perfusate because of their immiscibility and because, owing to their volatility they would be rapidly blown off via the oxygenator. So in the absence of suitable equipment to deliver known quantities of the vapours, the perfusate was saturated by passing the O_2/CO_2 (95:5) mixture through the liquid anaesthetic, as described for administration to the animals, and then into the perfusate oxygenator for 10 min. If it is assumed that the solubilities of the anaesthetics in Krebs–Ringer bicarbonate are similar to those in water[10,11] their perfusate concentrations for the 10 min of administration will be considerably higher than in rat blood during surgical anaesthesia; about fifteen to twenty times for halothane[12] and about five times for trichloroethylene (extrapolating from data for humans[13]; no values are available for rats). After the period of saturation the anaesthetics are removed rapidly via the oxygenator.

All the anaesthetics were added to the perfusate after 90 min of blank perfusions—that is, with nothing added to the perfusate—and the perfusions were continued for a further 90 min. Thus each perfusion consisted of an experiment, together with its own blank. This avoided the statistical difficulties involved in running a series of perfusions with and without added anaesthetic, reduced the number of perfusions required and removed the need for statistical analysis of the results. To make results within each experiment comparable, and to avoid any possible effects of using different anaesthetics together in one experiment, the same one was used for the operation for

removal of the liver from the donor rat as was added to the perfusate for the experiment.

Whole animal experiments

Rats of the same weight and type as used in the perfusion experiments were kept under halothane or trichloroethylene anaesthesia for 30 min. Approximately 5 ml of blood was withdrawn by cardiac puncture with a siliconized syringe, heparinized and immediately centrifuged at 4° to remove cells. The plasma was stored at -30° till required for assay.

Immediately after withdrawal of blood, livers were removed and treated as described below for liver water and potassium determinations.

Chemical estimations

Liver water and potassium. Livers from control animals were quickly removed under pentobarbitone anaesthesia, perfused free of blood with saline and the left lobe removed, weighed and dried to constant weight at 110° (about 72 hr). Livers from perfusion experiments were treated in the same way but without prior perfusion with saline. Samples of the dried material were digested in a nitric acid/sulphuric acid mixture and the potassium content measured by flame photometer.

Perfusate potassium. Perfusate samples were diluted 1:50 and read directly by flame photometer.

Perfusate urea and ammonia. Urea was measured by the method of Fawcett and Scott[14] which involves the determination of ammonia, after incubating with urease, by the phenate-hypochlorite colourimetric method. Free ammonia was measured in the same way but omitting the urease incubation. Concentrations of samples were such that there was no inhibition of colour production by protein, so that initial separation of the ammonia by diffusion was not necessary. Each of the anaesthetics was tested and found not to inhibit colour production at the concentrations used.

Perfusate glucose. Glucose was measured by the method of Marks[15] without deproteinization of perfusate. None of the anaesthetics used interfered.

Perfusate solids. One millilitre of the perfusate remaining at the end of the perfusion was evaporated and dried to constant weight at 110°. After subtraction of the weight of solids from the original Krebs–Ringer bicarbonate buffer, and correction for evaporation the weight of substances given out by the liver during perfusion was calculated.

RESULTS

Perfusion experiments

The effects on liver metabolism of anaesthetics added to the perfusate during perfusion were tested by measuring changes in the rate of urea and glucose production, and the general condition of the liver was assessed by measuring changes in liver potassium levels, water content and bile secretion.

Urea production and perfusate ammonia levels. It is found that when nothing is added to the perfusate during a 3-hr blank perfusion urea is produced linearly throughout and perfusate ammonia remains below 0·2 mg NH_3-N per 100 ml. Pentobarbitone, thiopentone and tribromoethanol, when added to the perfusate, all caused significant inhibition of urea production and a rise in perfusate ammonia (Fig. 1). These effects

were more marked in the cases of thiopentone and tribromoethanol where there was complete inhibition of urea output at first, but signs of recovery from both after about 1 hr. Pentobarbitone, however, inhibited urea production by about 50 per cent but this inhibition was maintained for the rest of the perfusion. Faster recovery from the effects of the quicker acting anaesthetics may be expected because of the greater breakdown rate of these in the liver.[9,16]

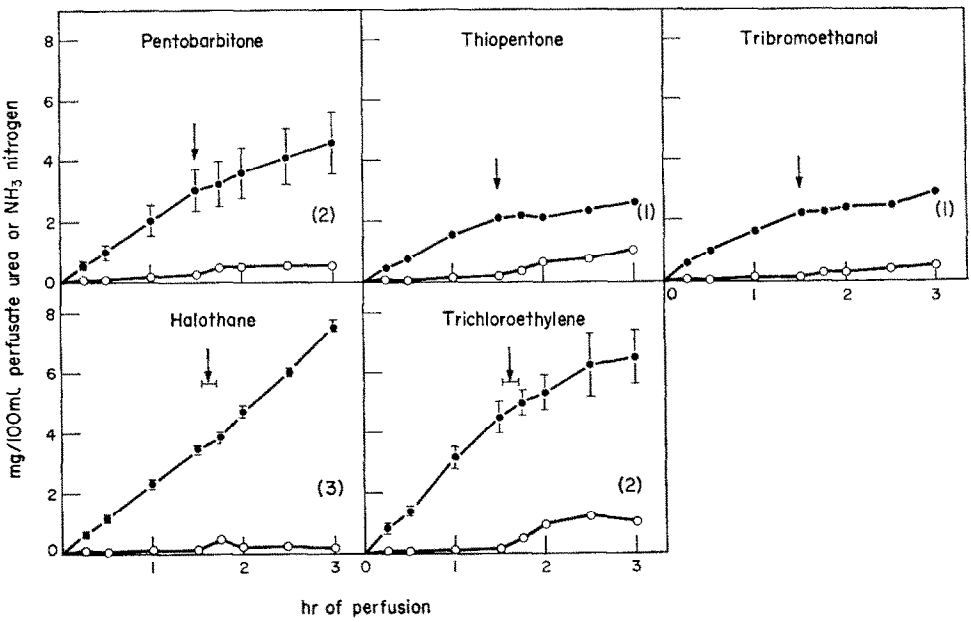

FIG. 1. Effect of the anaesthetics on urea production and ammonia levels. The arrows indicate time or duration of administration of anaesthetic. Bars indicate range, and figures in parenthesis the number of perfusions. —●— urea; —○— ammonia.

It should be noted that the lower initial rates of urea production (and also of glucose production, see below) in the thiopentone and tribromoethanol perfusions and one of the pentobarbitone perfusions imply that the first doses of the respective anaesthetics, i.e. for operation anaesthesia, were already having deleterious effects on liver metabolism before application of the booster doses to the perfusate.

Figure 1 shows that halothane had a transient effect on urea production which was reversed immediately after administration was stopped. Trichloroethylene produced a similar inhibitory effect, but an effect which was not corrected at once. In fact during the last half hour of these perfusions urea output had decreased even further, long after the trichloroethylene should have been "blown off" via the oxygenator.

In the same way ammonia levels are raised significantly but only transiently by halothane, but continued to rise after the end of the trichloroethylene treatment and remained elevated for the rest of the time.

Glucose production. During 3-hr blank perfusions, glucose is produced by the liver at a nearly constant rate, except where thiopentone and tribromoethanol are used for the operation.

Addition of pentobarbitone, thiopentone and tribromoethanol not only caused a total inhibition of glucose output (Fig. 2), but in the cases of thiopentone and tribromoethanol and in one of the pentobarbitone perfusions glucose was taken back into the liver, presumably in an attempt to overcome the respiratory block caused by the anaesthetics.[17,24]

Halothane produced a pronounced inhibition of glucose output (Fig. 2) while it was present in the perfusate but the inhibition tended to be reversed as soon as it was "blown off". As in the case of urea production the inhibitory effect of trichloroethylene increased for 20–30 min after administration, but there was some recovery during the last hour.

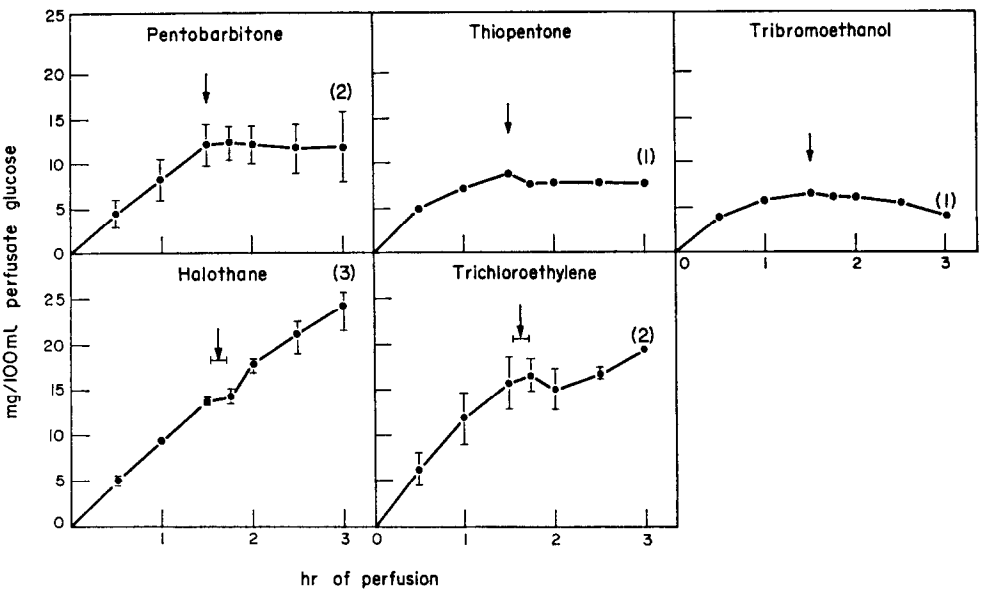

FIG. 2. Effect of the anaesthetics on glucose production. The arrows indicate time or duration of administration of anaesthetic. Bars indicate range, but are omitted where the range is less than the height of the symbol. Figures in parenthesis indicate the number of perfusions.

Perfusate potassium. Changes in potassium levels of the perfusate rather than changes in liver levels were measured because the loss of as little as 2 per cent is clearly seen as a rise in perfusate concentration, and because it provides a continuous monitor of the state of the liver throughout the perfusion. Figure 3 shows how perfusate potassium changes during blank perfusions. During the first half hour the liver rapidly regains the potassium ions it lost during the operation and the setting up of the perfusion; this manifests itself as a fall in perfusate level, and amounts generally to about 15 per cent of the normal liver content. Thereafter the perfusate potassium concentration remains constant, indicating that there is no subsequent loss from the liver as a result of hypoxia or damage from other causes. These perfusions were run for 2 hr but identical results are obtained with 3-hr perfusions. As well as giving a good guide to the condition of the liver during the perfusion, the rate and amount of initial potassium recovery also give some information about how the liver fared during

the operation. Measurement of liver potassium at the end of perfusion indicates that when pentobarbitone or halothane is used for the operation this initial potassium recovery approaches 100 per cent.

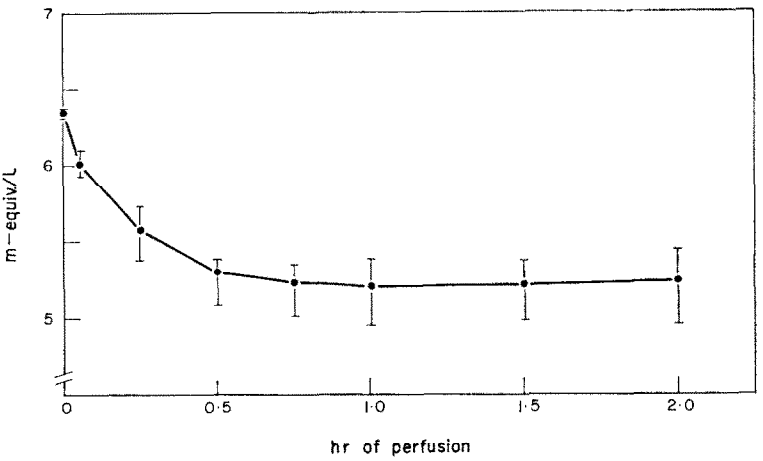

FIG. 3. Changes in perfusate potassium concentration during blank perfusions. Pentobarbitone was used as anaesthetic for removal of the liver in these perfusions. The bars indicate the range over four perfusions.

Addition of pentobarbitone to the perfusate resulted in an immediate rise in potassium ion concentration caused by a loss from the liver (Fig. 4). This leakage of intracellular potassium slowed down and stopped after about 15 min, when about 7 per cent of the normal (control) liver level had been lost, but there was little sign of much of this potassium being recovered during the remaining hour.

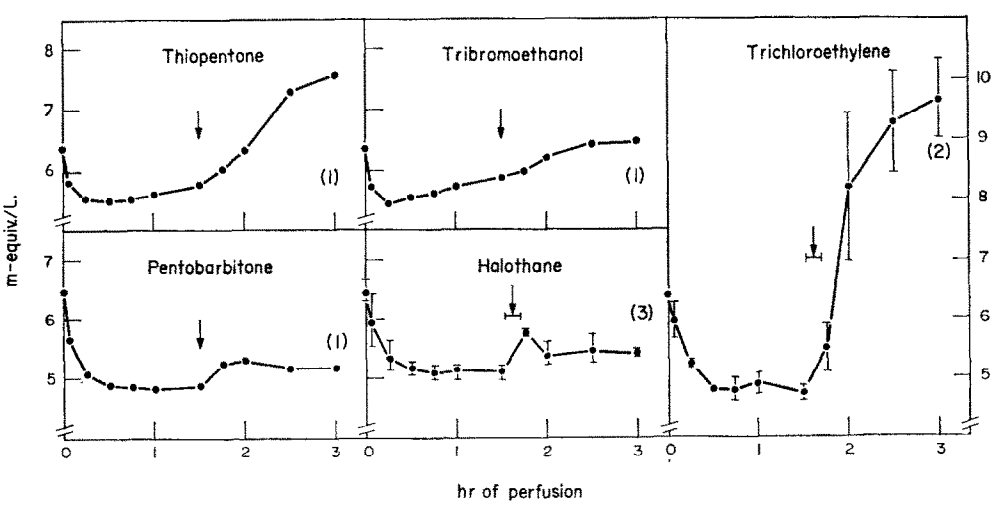

FIG. 4. Effect of the anaesthetics on perfusate potassium levels. The arrows indicate time or duration of administration of anaesthetic. Bars indicate range, but are omitted where the range is less than the height of the symbol. Figures in parenthesis indicate the number of perfusions.

In the cases of thiopentone and tribromoethanol the return of potassium to the liver during the first 30 min of perfusion amounted to much less than with the other anaesthetics. During the next 30–90 min this recovery was reversed and the liver lost potassium, about 4 per cent in the thiopentone perfusion and 7 per cent in the tribromoethanol perfusion, so that probably the initial uptake did not amount to a complete recovery after the operation. Thus the livers in these experiments were showing signs of deterioration even before addition of the booster dose, which then caused a further loss of potassium from the cells of 40 per cent in the case of thiopentone and 15 per cent in the case of tribromoethanol. Measurement of the potassium contents of the livers at the end of perfusion confirmed that considerable quantities were lost during the respective operations which were not recovered at the beginning of the perfusions (about 16 per cent in the thiopentone experiment and 10 per cent in the tribromoethanol experiment).

Halothane (Fig. 4), when added to the perfusate, and while still present, caused a small but significant loss of liver potassium (ranging in the three perfusions from 8 to 11 per cent of control levels), but half of this was recovered as soon as administration of anaesthetic was stopped. However, there remained a residual 3–6 per cent which was not regained during the final hour's perfusion. Measurement of liver potassium at the end of the perfusion indicated that all the potassium lost during the operations was recovered in the first 30 min in each of the three perfusions.

The greater uptake of potassium at the beginning of the trichloroethylene perfusions compared with that of the halothane perfusions, implies that more potassium was lost during the operation in the trichloroethylene experiments than in the halothane experiments. If it is assumed that in each case all the potassium lost during the operations was recovered, the trichloroethylene livers lost about 16 per cent more potassium during the operation, on average, than the halothane livers. In view of the small number of perfusions one cannot say whether this is a consistent difference, but the results of the whole animal experiments support this idea (see below).

The effect of trichloroethylene on the perfused liver was more marked; addition to the perfusate caused a great and rapid leakage of intracellular potassium into the medium. At the end of two perfusions one of the livers had lost 70 per cent and the other 90 per cent of its potassium, and leakage was still continuing in both cases, without any sign of recovery.

Liver water. Table 1 shows that treatment of the perfused liver with each of the anaesthetics under consideration caused oedema to some extent as compared with livers perfused for 3 hr in blank experiments (using pentobarbitone as operational anaesthetic) and controls (non perfused livers). The extent of water uptake was not great except in the trichloroethylene-treated livers which were massively oedematous.

The weights after perfusion of the trichloroethylene-treated livers were well below the range of the blank perfused livers, indicating that they had lost a considerable proportion of their (dry) weight during perfusion. This was confirmed by measurement of perfusate solids, from which it was calculated that the initial weights of the two livers were 1·98 and 2·22 g, values within the range of the unperfused controls.

Bile production (Table 2) was severely inhibited by pentobarbitone, thiopentone, tribromoethanol and trichloroethylene, but only slightly so by halothane (rate reduced by 13 per cent on average). The livers from thiopentone, tribromoethanol and

one of the pentobarbitone perfusions produced very little bile even before the second, booster, dose.

Whole animal experiments

No significant difference was found in blood ammonia or liver water of rats kept under surgical halothane or trichloroethylene anaesthesia for 30 min. Plasma potassium

TABLE 1. EFFECT OF ADDED ANAESTHETICS ON WATER CONTENT OF LIVERS AFTER PERFUSION

Anaesthetic added to the perfusate	Dry weight of livers after perfusion (g)	Per cent water content (by weight)	Water/solid (weight) ratio
Unperfused controls	2·29 ± 0·17 (15)	72·4 ± 1·6	2·59 ± 0·24
	range	range	range
	1·90–2·57	69·7–75·2	2·18–3·03
3-hr Blank perfusions*	2·01 ± 0·12 (15)	73·5 ± 0·9	2·77 ± 0·13
	range	range	range
	1·82–2·24	72·4–75·3	2·61–3·03
Pentobarbitone	2·25	78·8	3·73
	2·04	71·9	2·56
Thiopentone	2·17	74·6	2·93
Tribromoethanol	1·91	74·2	2·96
Halothane	2·38	74·2	2·88
	2·25	74·3	2·90
	2·17	74·8	2·97
Trichloroethylene	1·49	89·6	8·53
	1·57	90·7	9·71

Figures in parenthesis indicate the number of experiments. See text for amounts of anaesthetics added and conditions.

* Pentobarbitone was used for the operation. No anaesthetic was added to the perfusate.

TABLE 2. EFFECT OF ADDED ANAESTHETICS ON BILE PRODUCTION

Anaesthetic added	Rate before addition (ml/hr)	Rate after addition (ml/hr)
Pentobarbitone	0·54	0
	0·07	0·04
Thiopentone	0·10	0·09
Tribromoethanol	0·19	0·02
Halothane	0·57	0·37
	0·68	0·58
	0·37	0·47
Trichloroethylene	0·52	0·08
	0·44	0·24

levels could not be compared because trichloroethylene caused severe haemolysis, but the liver potassium concentrations of trichloroethylene-treated rats were slightly but significantly lower than the levels of those treated with halothane (see Table 3).

DISCUSSION

Experiments with animals *in vivo* and with liver preparations *in vitro* have shown that all the anaesthetics studied here are metabolized to varying extents by the

liver,[5,9,18–23] but there is little known about their effects on other metabolic reactions in the liver. Compared with whole animal studies the perfused organ has the advantage that secondary metabolic changes caused by effects on hormone production or on other tissues are not present to complicate the picture, and it is interesting that each of the anaesthetics investigated had some direct effect on the state of the perfused liver. A disadvantage of the perfusion experiments, however, is that the liver

TABLE 3. LIVER POTASSIUM LEVELS IN RATS DURING HALOTHANE AND TRILENE ANAESTHESIA

Anaesthetic used	No. of experiments	m-equiv. Potassium per 100 g dry weight		
		Mean	S.D.	Range
Halothane	6	35·9	±1·6	34·9–38·9
Trichloroethylene	6	31·8	±1·8	29·5–34·5

$P < 0{\cdot}002$.

The animals were kept under surgical anaesthesia for 30 min before removal of livers for potassium analysis (see Methods).

has been subjected to the same anaesthetic during the operation, so that the anaesthetic added to the perfusate is a "booster" dose, the magnitude of whose effect will depend to some extent on how much the liver has recovered from the first dose.

Metabolic effects of the anaesthetics

Glucose production. During the operation and setting up of the perfusion the liver loses virtually all its glycogen and there is no re-deposition of glycogen stores during the perfusion[4] as is sometimes the case with livers perfused with blood or highly fortified media. The glucose given out by the liver therefore comes from direct synthesis and not from glycogen stores.

Pentobarbitone, thiopentone and tribromoethanol all markedly inhibited net glucose output and in many cases glucose was actually taken back by the liver. This, taken together with the known inhibitory effect of barbiturates on the respiratory chain (see for instance Brody;[17] Aldridge[24]) suggests that glycolysis is increased to overcome the loss of energy caused by the respiratory block. An increase in lactate concentration would be expected on this hypothesis, and although lactate was not measured here, Booker *et al.*[25] have shown a rise in blood lactate level during thiopentone anaesthesia in dogs.

In conditions where glycolysis is stimulated and the respiratory chain is inhibited an increased level of reduced pyridine nucleotides is to be expected. This, in turn, would lead to an increase in lactate and glutamate concentrations, for instance, at the expense of pyruvate and α-oxoglutarate and, indeed, a fall in blood pyruvate and α-oxoglutarate levels in pentobarbitone-anaesthetized rats and rabbits has been shown by Goodwin and Williams.[26]

No fall in blood glucose,[27–30] and in some cases an actual rise[25,28] has been shown in various animals under barbiturate anaesthesia. If, as indicated here, net glucose synthesis in the liver is inhibited, the blood levels in the whole animal experiments must be maintained either from liver glycogen stores or by impairment of glucose

utilization by the peripheral tissues, or both; and recent work by Dulin[31] has shown that the latter is the case in dogs anaesthetized by the barbiturate Cyclopal sodium, and that it is at least in part due to a marked inhibition of glucose transport into muscle, and to a lesser extent into brain.

The effect of halothane on glucose production was only temporary even at the high concentration used, and that of trichloroethylene was more pronounced and complex, but too little is known about metabolic effects of these anaesthetics for any comment to be made on these results.

Urea production. All the anaesthetics inhibited urea production by the liver, though the effect of halothane was transient and small when the size of the dose is taken into consideration. The inhibition in each case was accompanied by a rise in perfusate ammonia suggesting (but not conclusively showing) that the effect of the anaesthetics was not merely on the transport of urea out of the liver cells, but on its synthesis. This is also suggested by the observation of Booker *et al.*[25] that urea production from amino acids was impaired during prolonged thipentone anaesthesia in dogs.

It is interesting to note that the greatest increases in perfusate ammonia followed the administration of trichloroethylene and thiopentone. Symptoms such as nausea, convulsions, lethargy and others are associated with recovery from large doses of these anaesthetics in particular. It is tempting to speculate whether these effects could be due to a raised ammonia level in the brain, since superficially similar symptoms are observed in conditions in which blood ammonia is raised, though not necessarily from generalized liver damage. (See for instance, Bessman,[32] Russell *et al.*,[33] Kirk and Sumner.[34])

Liver damage

The criteria mentioned previously—loss of potassium, water gain, inhibition of bile production and also changes in flow rate of perfusate through the liver—indicate that in most cases the anaesthetics caused damage to the liver other than the effects on its metabolism described above. Of the anaesthetics studied, halothane at a perfusate concentration fifteen to twenty times blood levels during deep anaesthesia, caused minimal damage. In contrast, trichloroethylene, at five times the deep anaesthesia level, produced extensive damage.

The relatively slow loss of potassium by the liver after addition of the barbiturates and tribromoethanol indicates that the damage from these anaesthetics is a secondary effect, possibly from inhibited respiration suggested above.

The outstanding difference between halothane and trichloroethylene in these perfusion experiments was that the effects caused by halothane were reversed immediately, while those of trichloroethylene continued and increased well after most of it should have been removed via the "lung" of the system. By the end of the trichloroethylene perfusions the livers had lost nearly all their potassium, and in spite of a doubling of their wet weights they had lost up to 30 per cent of their dry weights. A third perfusion had to be stopped 45 min after trichloroethylene administration because massive oedema stopped perfusate flow. This is clearly a result of damaged membranes or membrane functions, and this idea is supported by the fact that severe haemolysis was observed in blood of whole rats after anaesthetization for 30 min.

Rees[35] has suggested that the two major characteristics of liver damage—fatty liver and necrosis—are separate events, resulting from two distinct sets of reactions.

He also suggests that permeability changes, such as potassium leakage, are involved in the pathway leading to necrosis and ultimately autolysis but not to fatty changes. If this is correct, the potassium measurements in the perfused liver suggest that trichloroethylene, in large quantities, possibly will cause liver necrosis in rats but that halothane does not, as least at concentrations up to twenty times the levels found during deep anaesthesia. This view is supported by the work of Jones *et al.*[36] who showed that in mice trichloroethylene can cause necrosis whilst large doses of halothane produce changes such as fatty liver but no liver necrosis. Norris *et al.*[37] were also unable to demonstrate necrosis in livers of dogs after repeated exposure to halothane. These results, in addition, demonstrate again that fatty liver can be produced in the absence of necrosis.

Further evidence that halothane does not disturb ion transport at the cell membrane comes from the work of Snodgrass and Piras[38] who observed no increase in mitochondrial calcium, to be expected[39–41] if calcium had leaked from the extra- to the intra-cellular fluid, after administration of halothane to rats. This contrasts with a marked increase after administration of carbon tetrachloride.[39,40,42] Judah *et al.*[41] have suggested that this calcium uptake by the mitochondria is involved in the development of necrosis.

Thus, although from their physico-chemical natures both halothane and trichloroethylene might be expected to have a direct effect on cell membranes[43] the results from the perfusion experiments together with the results of other workers discussed above show that their effects on the liver are quite different. Trichloroethylene may belong to the group of necrogenic agents which include chloroform and carbon tetrachloride, though direct comparison shows trichloroethylene to be less damaging than chloroform;[36,44] halothane clearly cannot be placed in this class in spite of clinical similarities between halothane and chloroform.[45] This distinction is important because trichloroethylene is still sometimes used as an anaesthetic in cases with a history of liver disease in the belief that it is less hepatoxic than halothane.

Halothane caused least damage of all the anaesthetics studied and for perfusion experiments is the anaesthetic of choice because of its small effect on metabolism, its non necrogenic properties and its rapid removal from the system by ventilation.

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