LACK OF TOXICITY OF HALOTHANE ON DIFFERENTIATED LIVER CELL CULTURES

BY

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SUMMARY

Differentiated human embry liver cell cultures were exposed for varying periods of time to halothane–nitrous oxide–oxygen mixtures and also to 1 per cent and 5 per cent chloroform vapour. No morphological changes were found after exposure to halothane. Exposure to chloroform was rapidly followed by fatty degeneration and complete cell necrosis.

Halothane is now the commonest vapour in anaesthetic gaseous mixtures in general use in this country and elsewhere. Considerable literature has accumulated concerning the possible association between halothane and postoperative hepatic necrosis. Retrospective studies on halothane toxicity in man (Mushin et al., 1964; Slater et al., 1964; and the American National Halothane Study, 1966) have not established a causal relationship between halothane and hepatic necrosis, though none of these studies completely excludes the possibility. It also remains to be shown that there is no connection between liver changes and repeated administration of the anaesthetic.

Recently Zuckerman, Tsiquay and Fulton (1967) described a method for the tissue culture of differentiated parenchymal liver cells using human embryo liver. It was thought, therefore, of considerable importance to examine the effect of halothane on human hepatocytes. There has been a previous report that halothane was without effect on the Chang cells (Corssen, Sweet and Glenweht, 1966), but these cells are not regarded as normal parenchymal liver cells since the cells developed the ability to produce a new growth and have undergone transformation; indeed, Moore, Southam and Sternberg (1956) were unable to distinguish the Chang cells cytologically from known tumour cell lines.

MATERIALS AND METHODS

Livers were obtained by the Tissue Bank of the Royal Marsden Hospital, after hysterotomy, from human embryos free from infection and abnormalities. The livers were cultured by the method described by Zuckerman, Tsiquay and Fulton (1967). The liver cell cultures from three different embryos were placed in a vacuum desiccator which was evacuated twice to $-22$ mm Hg at room temperature (fig. 1). The desiccator was then refilled with 1 per cent halothane in a mixture of 2 parts nitrous oxide and 1 part oxygen, delivered from a Boyle's anaesthetic machine. The cultures in the desiccator were then incubated at a constant temperature of $35^\circ$C throughout the period of the experiments. Times of exposure varied from 2 to 24 hours continuous exposure. Other cells were exposed twice and three times to repeated 2-hour exposures after intervals of overnight growth in normal atmospheric conditions with 2 per cent added carbon dioxide. Cell cultures, exposed continuously to mixtures of 1 per cent and 5 per cent chloroform vapour were used in parallel for comparison, and cells not exposed to any anaesthetic gases as controls. Preparations were examined at intervals by fluorescence microscopy after staining with acridine orange and by phase-contrast illumination. A number of preparations were subsequently maintained in culture for seven days (table I).

RESULTS

After exposure to halothane continuously for 2 to 24 hours the liver cells remained completely normal (fig. 2) and no morphological changes were detected in the nucleus nor in the cytoplasm throughout the whole seven-day period of
A confluent monolayer of human embryo liver cells after 40 hours in culture. ×500

The appearance of cultured liver cells after continuous exposure for 24 hours to 1 per cent halothane in a mixture of 2 parts nitrous oxide and 1 part oxygen. The cells are completely normal. ×500

A culture of liver cells after exposure to 1 per cent chloroform vapour for 24 hours. There is complete cellular necrosis. The nuclei are opaque with total loss of all morphological features and there is marked cytoplasmic fatty necrosis. ×500

**Table I**

*Number of differentiated liver cell cultures used and exposure times to the anaesthetic gases.*

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>Halothane</th>
<th>Chloroform</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single exposure</td>
<td>Repeated exposure</td>
<td>1% concentration</td>
</tr>
<tr>
<td>2 hours</td>
<td>10</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>4 hours</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>24 hours</td>
<td>8</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>7 days</td>
<td>8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>32</td>
<td>20</td>
</tr>
</tbody>
</table>
obtained. Repeated exposure to halothane at
intervals likewise revealed no difference from the
control cultures.

Cell cultures exposed to both 1 per cent and
5 per cent chloroform vapour showed some fatty
cytoplasmic necrosis after 2 to 4 hours and com-
plete cellular necrosis after 18 hours exposure
(fig. 3).

DISCUSSION

Animal experimentation supports the conclusion
that halothane in inspired concentrations of 1 per
cent does not produce liver cell changes. Bloxam
(1966) showed that in the perfused rat liver,
halothane, in contrast to other anaesthetics, pro-
duces no biochemical changes. Middleton and
associates (1966), using the isolated perfused
bovine liver, found that only high concentrations
of halothane 5-6 per cent in the perfusion fluid
for a period of 3 hours caused both biochemical
and structural changes in the liver. It is not clear,
however, how these concentrations of halothane
were achieved in the isolated perfused liver. If
3 per cent halothane was employed no demons-
trable biochemical changes occurred. In the third
of their experiments the calves were anaesthetized
using open drop halothane for induction, and
0.5-1.0 per cent halothane in oxygen to maintain
anaesthesia for 1 hour one week before perfusion.
The animals were allowed to recover and a week
later their livers were perfused using 3 per cent
halothane for 3 hours. The procedure seemed to
produce structural changes in the endoplasmic
reticulum and mitochondria and the authors con-
cluded that repeated exposure to halothane might
potentiate this effect. Snodgras and Pinas (1966)
reported that the in-vitro addition of high con-
centration of halothane to rat mitochondria pro-
duced metabolic changes, although mitochondria
isolated from the livers of rats which had been
previously exposed to halothane were found to be
metabolically intact.

There is an important limitation in animal
experimentation on the action of toxic substances,
in that there are marked species specificity of
toxic agents, and halogenated hydrocarbons are
no exception to this rule (von Oettingen, 1964;
Slater, 1966). The study of the exposure of
human liver cells to halothane is therefore of
some significance.

These results are clear-cut in so far that no
alterations in cellular morphology were detected
after exposure to the usual halothane gaseous
mixture employed for general anaesthesia. If
halothane is detoxicated by microsomal enzyme
systems it would be expected that these human
embryonic liver cells might be even more sensi-
tive to this vapour, since animal experiments
indicate that foetal liver has a lower level of drug
detoxication ability than the adult liver (Dallner,

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REFERENCES

Bloxam D. L. (1966). Effects of halothane and tri-
chloroethylene on rat liver. Lancet, 2, 1080.

Corssen, G., Sweet, R. B., and Gienweht, M. B.
(1966). Effects of chloroform, halothane and
methoxyflurane on human liver cells in-vitro.
Anesthesiology, 27, 155.

Dallner, G., Schiewitz, P., and Palade, G. E.
(1965). Synthesis of microsomal membranes and their
enzymatic constituents in developing rat liver.

Kato, P., Vassanelli, P., Frontino, G., and Chiesara,
A. (1964). Variation in the activity of liver micro-
somal drug metabolising enzymes in rats in relation
to the age. Biochem. Pharm., 13, 1037.

Middleton, M. D., Roth, G. F., Smuelker, E. A.,
and Nyhus, L. M. (1966). The effects of high concen-
tration of halothane on the isolated perfused

Moor, A. E., Southam, C. M., and Sternberg, S. S.
(1956). Neoplastic changes developing in epithelial
cell lines derived from normal persons. Science,
124, 127.

Mushin, W. W., Rosen, M., Bowen, D. J., and Camp-
bell, H. (1964). Halothane and liver dysfunction:
a retrospective study. Brit. med. 7., 2, 329.

von Oettingen, W. F. (1964). The Halogenated Hydro-
carbons of Industrial and Toxicological Importance.
New York: Elsevier.

Slater, E. M., Gibson, J. M., Dykes, M. H. M., and
Walzer, S. G. (1964). Postoperative hepatic
necrosis: its incidence and diagnostic value in
association with the administration of halothane.


of halothane on rat liver mitochondria. Bio-
chemistry, 5, 1140.
BOOK REVIEW


This book brings together in convenient form aspects of anaesthesia which have particularly interested the author. It is divided into six parts dealing with uptake, elimination and potency of the inhalational anaesthetics, with nitrous oxide, intravenous anaesthesia in the dental chair, fainting, vomiting, and the teaching of dental anaesthetics. As he makes clear himself, all the chapters except the one on intravenous anaesthesia in dentistry, which occupies half the book, have appeared in some form already.

Although the rest of the book still makes interesting reading the most important pages are those concerned with intravenous techniques in dental anaesthesia, especially as these are not yet fully covered in conventional anaesthetic textbooks. This section is devoted mainly to the use of methohexitone in the dental surgery. That dentists are included among intended readers is indicated by the detail with which venepuncture is described. There is an excellent description of anaesthetic technique in which the use of propofol and of Jorgensen’s method of sedation are also covered. Readers will not be surprised at Dr. Bourne’s insistence that patients should always be anaesthetized lying down, in view of his well-known work on the risks of unsuspected hypotension occurring in the upright position. Dentists who have accustomed themselves to operating upon supine patients find it more natural and less tiring. Complications and contraindications are fully discussed, but some readers may feel that insufficient stress is laid upon the limitations of methohexitone alone for conservation. In the reviewer’s experience, where it works well it is impressive but in some patients it produces restlessness which makes accurate dentistry very difficult. In his description of the Jorgensen technique it is surprising to read that he condones the practice of allowing a full meal beforehand, especially when on the next page he indicates that vomiting may occur. Another point of criticism, particularly relevant as dentists will use this book as a guide, is his attitude towards the diabetic, whom most anaesthetists would surely prefer to see in hospital.

The last chapter is the paper read by Dr. Bourne at a symposium on the teaching of general anaesthetics for dental surgery held in 1964 at the Royal College of Surgeons in London. He argues that dentists, who are keen to learn, should be instructed in dental anaesthetics for the practical reason that they have to give them. In his experience doctors show little enthusiasm for this branch of the specialty. Many anaesthetists are unhappy about teaching dentists but others will agree that the author is taking a realistic view of the present situation. He then discusses the growing use of methohexitone in conservative dentistry. He believes that this movement has already gained such momentum that nothing would now stop it. He supports the concept of the operator-anaesthetist in this field, taking the view that there is no other solution in the state of medical (and dental) manpower today. Most anaesthetists, and many dentists, do not accept this analysis of the situation, suggesting rather that it is being brought about primarily by the activities of a group of dentists who take the view that all patients should be sedated or unconscious for conservation. Intravenous techniques in dentistry are certainly a major advance but there are genuine reservations for them. There is no justification for their routine use, especially when this forces the operator to act as his own anaesthetist. Standards of anaesthesia in this country have reached such a high level that nothing short of complete safety and perfect operating conditions should suffice, and these are not guaranteed by methohexitone and the operator-anaesthetist. In a final addendum the author suggests that the manpower problem might be got over in the future by employing paramedical anaesthetists in the dental surgery; these could work also in hospital under consultant supervision. One can only hope that this represents an unduly pessimistic view of our specialty’s prospects.

Dr. Bourne is an authority on his subject. His book, which contains a comprehensive bibliography, is thoroughly informative and gains from the well-argued expression of some controversial and unconventional opinions. Anyone interested in modern dental anaesthesia should not fail to read it.

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