EFFECTS OF ANESTHETIC AGENTS ON HEPATIC STRUCTURE AND FUNCTION IN DOGS

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THE PROBLEMS of anesthesia in mass casualty care are largely unsettled and solutions proposed remain speculative.[1] Envisioning the wholesale employment of potent anesthetic agents by nonmedical personnel, attempts are being directed toward formulating doctrines which will insure simplicity of administration of anesthetic agents and minimize the inherent dangers.[2] As a part of a study on anesthesia in mass casualty care, the hepatic effects of chloroform, diethyl ether and Fluothane were investigated. These inhalants represent three of the potentially useful agents satisfying mass casualty requirements. The effects on the liver were to be studied simultaneously by histopathologic evaluation and by a test of hepatic function utilizing radioactive iodine-tagged rose bengal. A critical evaluation among the different anesthetic agents was then to be attempted.

The hepatotoxic effects of chloroform are well known and have been a deterrent to its widespread use in this country. Most of the reports dealing with the injurious effects of chloroform on the liver have dealt with frank poisoning, and with its utilization in light or deep anesthesia. The usefulness of this agent in analgesic concentrations is largely unexplored. In this study we have attempted to compare analgesic with surgical anesthetic effects.

Descriptions of the hepatic morphologic changes induced by chloroform toxicity in humans and in experimental animals usually stress centrolobular necrosis and fatty changes as representative of the acute phase of injury and parenchymal regeneration and fibrosis as chronic phase phenomena.[3, 4, 5] In experimental animals, damage is roughly proportional to the dosage. It is of note that variations in species susceptibility is well established and that susceptibility of a given animal may be modified by diet, infection, anoxia and stress.[6-16]

Contrary to most reports on the subject of chloroform hepatoxicity, Orth and colleagues found minimal abnormalities in liver biopsy studies and bromsulfalein excretion tests in dogs and slight, transient abnormalities in liver function tests in humans.[17]

In dogs ether anesthesia is capable of producing mild anatomical injury [18] and transitory liver function impairment.[19] Fairlie *et al.* found mild, transient abnormalities in serial liver studies in patients with antecedent normal hepatic function [20] while French and coworkers reported more frequent, intense, and prolonged depression of liver function by ether in patients with pre-existing liver disease.[21]

Limited studies of Fluothane by British investigators have indicated superior qualifications as an inhalant. Raventós reports no significant bromsulfalein and hippuric acid excretion abnormalities in one dog and four rats, respectively. Histologic evaluation of liver sections from rats, dogs and monkeys disclosed changes which were "of trivial extent and degree compared with changes known to occur in man after chloroform anesthesia." [22] In Johnstone's evaluation of Fluothane anesthesia, hepatic effects were not studied, but there was no clinical evidence of hepatic dysfunction or aggravation of existing liver disease and no hepatic lesions were described in four autopsies reported.[23]

Nonradioactive rose bengal dye excretion has been used as a hepatic function test for years.[24-27] Taplin *et al.* reawakened interest in the dye after tagging the rose bengal molecule with radioactive iodine (I^{131}) and using an external *in vivo* counting technique.[28] Taplan and

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coworkers and Mendeloff have shown that the reticuloendothelial system plays no role in uptake or excretion of the dye and concluded that the parenchymal cells of the liver were responsible for these activities.[29, 30] Cohen *et al.* demonstrated that the blood clearance curves for rose bengal and bromsulfalein were nearly identical in normal humans.[31] Simpson and Saperstein showed that rose bengal administered intravenously to dogs exhibited plasma volume distribution similar to Evans blue dye and that plasma clearance was complete within nine minutes after injection. Furthermore, they demonstrated that over a wide range of plasma dye concentrations clearance appeared to be independent of concentration and that there was no evidence of extrahepatic clearance of the dye.[32]

Method

Twenty-five male mongrel dogs weighing between 9 and 24 kg. were studied. Animals were fed a standard diet once each morning and water was allowed *ad libiditum*. The animals were placed in the following categories: controls, 6; chloroform analgesia, 6; chloroform anesthesia, 6; Fluothane anesthesia, 4; ether anesthesia, 3. Radioactive rose bengal tests and needle liver biopsies were performed on each animal for control purposes at least 24 hours, and usually 72 hours or more prior to the initial exposure to anesthetic agents. (Results later designated RBC.) These procedures were repeated on the second (RB-1), seventh (RB-2), and ninth (RB-3) days postexposure. The control animals were treated in the same fashion except for subjection to "air anesthesia." No efforts were made to change or supplement diet or to treat any clinical abnormalities in the animals.

The morning meal was withheld for animals undergoing anesthetic procedures, and the food was subsequently offered to these animals following recovery from anesthesia. All animals were initially given 8 mg. of atropine sulfate intravenously. Succinylcholine hydrochloride in doses of 0.15–0.40 mg./kg. was then administered intravenously. After complete muscular paralysis was attained, a cuffed endotracheal tube was introduced under direct vision and artificial ventilation begun by means of a Bird respirator pump or a Starling respirator. The cuff was inflated and the tidal volume adjusted for the animal. Scalp wire electrodes were placed for electroencephalographic recording using four leads: fronto-occipital, fronto-parietal, parieto-occipital, and bi-parietal on an eight channel Grass encephalograph (model 3D).

After a period of stabilization, the anesthetic agent was introduced by connecting the intake tube of the respirator to a Tecota or modified Duke inhaler for chloroform or Fluothane inhalation. Ether was administered with a closed circle carbon dioxide absorption system and pulmonary ventilation was provided by intermittent manual compression of the rebreathing bag.

Changes in electroencephalograph patterns during inhalation were analyzed visually. The electroencephalographic classification as described by Pearcy *et al.* for chloroform and Fluothane [33] and that outlined by Courtin for ether anesthesia [34] were used. Muscular paralysis and artificial ventilation using room air ("air anesthesia") were maintained for two hours in the six control animals.

Chloroform was administered to 6 animals in concentrations sufficient to produce level I or II electroencephalographic patterns. This was interpreted as analgesia. In the 6 animals representing light anesthesia, chloroform was given in concentrations sufficient to effect level III or IV patterns. Light Fluothane anesthesia was determined by level II electroencephalographic pattern, and light ether anesthesia was determined by level III or IV electroencephalographic patterns in the Courtin classification.

The analgesia or anesthesia was maintained for 90 minutes following which artificial pulmonary ventilation was continued until recovery from muscular paralysis was evident. All animals were returned to the postanesthesia recovery room for observation.

The apparatus utilized for the radioactive rose bengal determinations consisted of a collimated directional scintillation detector cabled to a count rate meter which in turn was attached to an Esterline-Angus recorder for continuous recordings (fig. 1). The assembly was allowed to operate for at least an hour to attain steady baseline tracings representing "background" radiation before the radioactive rose bengal procedure was started.

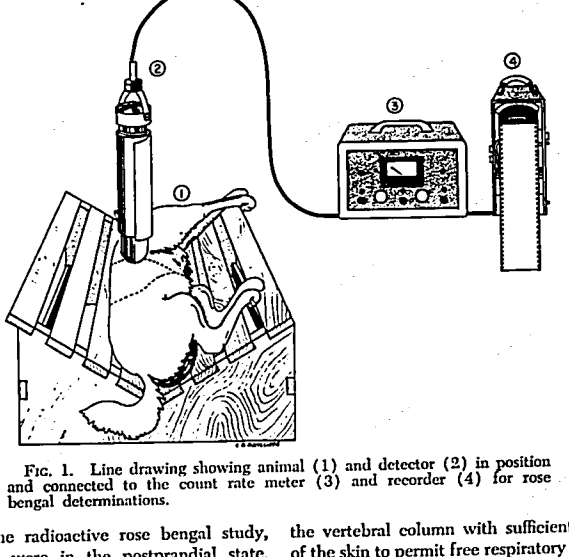

FIG. 1. Line drawing showing animal (1) and detector (2) in position and connected to the count rate meter (3) and recorder (4) for rose bengal determinations.

During the radioactive rose bengal study, the animals were in the postprandial state, three to eight hours following morning feeding. To insure immobilization of the animals, pentobarbital sodium, 12 mg. per pound of body weight, was given intravenously immediately before the test. This dose was approximately halved in the chloroform exposed animals because of the anticipated hepatic damage and dysfunction. Prompt anesthesia was thus accomplished, and it was seldom necessary to administer further doses to continue immobilization.

The anesthetized animals were placed in the left lateral decubitus position in a wooden trough constructed for the purpose. The detector suspended from a movable beam was then positioned over the previously shaved liver area. The detector was so aimed as to minimize or obviate interference by gall bladder, duodenum or hepatic flexure contents. Exact positioning of the detector was determined by each animal's bodily configuration but, in general, the instrument was placed below the upper border of liver dullness in the midaxillary line and angled slightly upward toward the lung and slightly backward toward the vertebral column with sufficient clearance of the skin to permit free respiratory excursions. Once positioning was achieved, the scintillation detector was fixed in place.

Ten microcuries of radioactive rose bengal (0.2 to 1.00 ml.) was given intravenously into a hind limb at a rate of approximately 0.1 ml. per second. Recordings were made for a minimum of 60 minutes and up to three hours in those animals exhibiting pronounced depression of dye excretion. After termination of recording, the detector was placed over the region of dye injection to detect possible extravasation, and then over the gall bladder, duodenal region to make certain that radioactive contents in these organs had not influenced the tracings obtained after detector fixation over the liver.

The rose bengal curves (fig. 2) were interpreted in a quantitative fashion according to the method devised by Gibson, Hurd, and Osborn [35] and by Lowenstein [36]: "At any time liver radioactivity (L) is equal to the difference between the amount of activity taken up (1-U) and the amount excreted (1-E), symbolically, $L = (1-U) - (1-E)$. Therefore, $L = E - U$. Both U and E decrease loga-

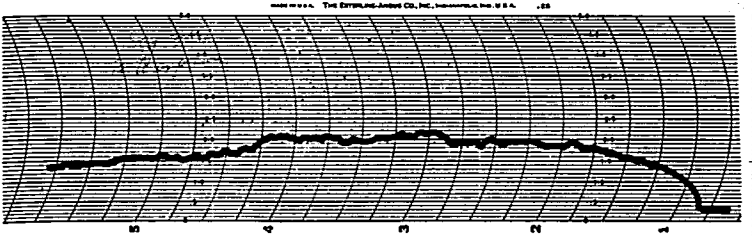

FIG. 2. The recording of a radioactive rose bengal determination in a normal animal.

rithmically with time; therefore, each of these components is characterized by a constant, which we may call uptake halftime (T_u) and excretion halftime (T_e), by analogy with the halflives of radioactive elements. The uptake halftime is the period in which the liver takes up half the circulating dye from the blood; the excretion halftime is the period in which half the amount taken up is excreted through the bile ducts. Each curve is analyzed as follows: after background activity is subtracted the curve is transposed to semilogarithmic paper (fig. 3); a straight line (E) is drawn from the ordinate through the descending limb —this is the excretion line; the uptake line (U) is obtained by plotting differences between E and the rising portion of the curve, and drawing another straight line from the ordinate through these points. The uptake halftime is the time when $U = \frac{1}{2}U$; the excretion halftime is the time when $E = \frac{1}{2}E$."

Immediately after the termination of the radioactive test, liver tissue was obtained by means of transcutaneous Vim-Silverman needle biopsy. Tissues were stored in buffered 10 per cent formalin solution (*p*H of 6.8–6.9) and the collected specimens ultimately were processed in two major lots. Stains employed included hematoxylin and eosin, periodic acid-schiff with salivary diastase controls for glycogen, and gallocyaninchrome alum (*p*H of about 1.0) for nucleic acids.[27, 28] These staining procedures were chosen to permit approximations of the gross derangements in carbohydrate, lipid, and protein metabolic activities.

RESULTS

Comparison of T_u and T_e values of the six controls prior to, and 2, 7, and 9 days post-exposure to "air anesthesia" with control values for each animal later subjected to some form of anesthesia showed no significant difference between the means of the groups. This indicated that no significant liver damage as measured by the rose bengal test was induced by the experimental method itself, *i.e.*, exposure to atropine and succinylcholine, pentobarbital sodium anesthesia, liver biopsy or

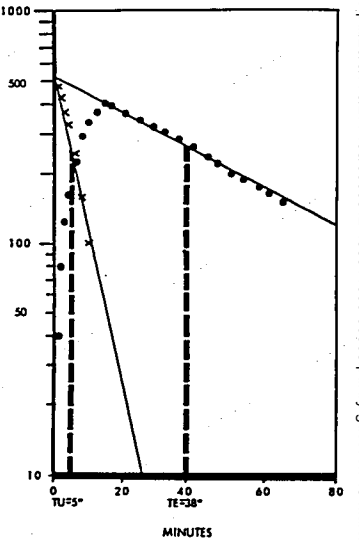

FIG. 3. The raw curve in figure 2 transposed to semilogarithmic paper for interpretation. Abscissa represents logarithmic value of counts per minute. (T_u, uptake halftime; T_e, excretion halftime.)

TABLE 1

T_u AND T_e VALUES (MINUTES) OF CONTROL GROUP AND INDIVIDUAL CONTROLS

RB-C		RB-1		RB-2		RB-3	
T_u	T_e	T_u	T_e	T_u	T_e	T_u	T_e
4.5	40	8	66	5.5	76	5.5	65
5	38	4.2	33	4	44.8	4	38
9	54	3.8	41	5	50	5.5	40
6	40	13	33	5	42	4	41
6	66	11	66	5	44	5.5	56
14	30	8	30	5.5	39	5	57

Chloroform Analgesia		Chloroform Anesthesia		Fluothane Anesthesia	
T_u	T_e	T_u	T_e	T_u	T_e
7	30	4.5	38	7.5	64
6	45	4.8	33	5.0	32
6.2	31	5	58	6	67
5.2	48	6	38	6.5	48

Comparison of T_u (uptake halftime) and T_e (excretion halftime) of control group with those of the individual controls in each group of animals receiving anesthesia indicated that repeated rose bengal test procedures did not alter uptake and excretion and that uptake and excretion of the dye was not significantly different in the control test of those animals who received anesthesia.

dietary factors (table 1). It is therefore valid to discard the group of six control animals, and to use each anesthetized animal's own control test as a control in the subsequent analysis.

Unfortunately, technical difficulties invalidated the rose bengal tests on the 3 animals exposed to ether anesthesia. There was no significant effect on T_u values of the other anesthetized animals. There was a highly significant difference between the T_e values for chloroform analgesia and chloroform anesthesia and between Fluothane anesthesia and either chloroform analgesia or anesthesia (table 2). Interpretation of these results indicates that (1) there is appreciable liver damage on the second post-chloroform day, as measured by the T_e values, and (2) that there is greater damage from chloroform employed at anesthetic levels compared with analgesic levels. More important, however, is the fact that liver function returned to levels not significantly different from normal by the seventh and ninth days post-exposure, under the conditions of this experiment, regardless of which agent was employed or what level of anesthesia was used.

No significant pathologic changes were found in the tissues obtained from the 6 control animals. Similarly, control specimens from all other animals presented no pathologic changes.

Liver biopsy sections obtained from the three other exposed animals and three of the Fluothane exposed animals showed "minimal histologic changes,"[30] *i.e.*, minimal, nonspecific pleomorphism, slight differences in cellular staining properties, rare minute foci of necrosis, and minimal, occasional pericholangitis. The sections from the fourth animal in the Fluothane group were not truly representative in that a previously biopsied site had been re-entered and inflammatory changes ensued which were reflected in subsequent biopsies.

Pathologic changes were found in all specimens other than controls from the chloroform analgesia and chloroform anesthesia dogs. Differentiation of the effects induced by analgesia from those of anesthesia could not be made with any degree of assurance because of overlapping histopathologic patterns between these groups. A greater range of variation, however, was discernible within the chloroform analgesia dogs than in the anesthesia animals, although this was not striking. Variations in both degenerative as well as regenerative phenomena was observed.

Among the earliest morphologic changes noted following chloroform were those of nuclear and cytoplasmic pleomorphism, loss of nuclear basophilia and cytoplasmic basophilia indicating nucleic acid depletions, irregular cytoplasmic basophilic clumping resulting in granular cytoplasmic appearances, and interpreted as indicating protein aggregation, and swelling to ballooning proportions of many cells in centrolobular zones. These ballooned cells often became vacuolated and almost transparent, lost their nuclei, and deposits of lipochrome pigment appeared. Glycogen was lost rapidly within these altered cells. Peripherally about these cells fatty changes were observed. Hyaline cytoplasmic accumulations were occasionally noted. Distinct autolytic cellular changes with fragmentation into eosinophilic bits followed by lysis were seldom seen in the sections from animals who did not succumb to anesthesia.

In contradistinction to general pathologic descriptions of chloroform hepatoxic effects, the animals in this study showed (1) *necrobiosis* rather than necrosis of the coagulative type and (2) relatively little fatty metamorphosis. Damage was confined to centrolobular zones of varying size and regularity which on occasion spread almost to periportal zones. The damaged areas appeared sharply delimited from adjacent essentially normal parenchymal cells. Only slight inflammatory infiltrations of neutrophils and mononuclear cells were encountered in damaged regions; mesenchymal reactive changes were mild; only minimal bile stasis was found.

The necrobiotic changes often resulted in "ghost cells" devoid of structural elements, but alignment and cohesion of cells conforming to cell plates (cords) were not dissociated. Replacement of these cells was prompt and was unaccompanied by inflammatory or noteworthy mesenchymal changes. Side by side with cellular degenerative changes active regenerative changes were usually apparent in the initial postexposure specimen. These regenerative changes were evidenced by cellular basophilia, particularly in perinuclear zones, polyploidy, pleomorphism within cells of youthful appearance and glycogen abundance. Amitotic regeneration was apparent and only rarely were mitoses found. In general, repair was well underway by the ninth postexposure day.

Three of the 4 animals dying after exposure to chloroform presented the characteristic histologic appearances of acute massive hepatic necrosis. No liver tissue was obtained on the fourth animal.

In order to attempt a statistically rigorous correlation between radioactive rose bengal test results and histopathologic evaluations over a nine day time span, the procedure of rank correlation was used.[40] Only those animals were utilized for correlation analysis for whom complete rose bengal test results with corresponding liver tissue sections were available. The original 25 dogs were reduced to 9 by virtue of: deaths before completion of the rose bengal tests, 4; technical inadequacy of rose bengal results in ether series, 3; incomplete biopsy series, 2; extraneous factors (infection) negating validity of biopsy series, 1; animals used as controls, 6.

By assessing all the morphologic changes held to be expressions of biologic significance a tabulation can be obtained of the severity of the changes produced by the anesthesia procedures. The ranking, from most severe to least severe damage, is largely based on degree, extent, and persistence of damage, and efficacy of regenerative responses toward control morphologic appearances. Little appreciable differences were found among the first

TABLE 2

COMPARISON OF T_u AND T_e VALUES (MINUTES) FOR ANIMALS EXPOSED TO THE INDICATED ANESTHETIC AGENTS

RB-C		RB-1		RB-2		RB-3	
T_u	T_e	T_u	T_e	T_u	T_e	T_u	T_e
Chloroform Anesthesia							
4.5	38	6.5	830	5.0	86	5.0	45
4.8	33	4.5	346	4.5	30	4.5	39
5.0	58	6.0	950	5.0	31	5.0	50
6.0	38	5.5	196	3.5	101	7.8	78
20.3	167	22.5	2322	18.0	248	22.3	212
Chloroform Analgesia							
7.0	30	22.0	270	6.2	64	7.0	37
6.0	45	10.0	118	7.0	34	6.8	43
6.2	31	10.0	138	5.0	101	4.2	50
5.2	48	10.0	98	7.0	57	8.0	53
24.4	154	52.0	624	25.2	256	26.0	183
Fluothane Anesthesia							
7.5	64	7.0	40	7.0	70	5.0	56
5.0	32	6.5	50	4.0	33	4.5	48
6.0	67	5.5	58	8.0	29	4.0	82
6.5	48	8.0	81	5.5	50	5.0	38
25.0	211	27.0	229	24.5	182	18.5	224

T_u = uptake halftime.
T_e = excretion halftime.
RB-C = rose bengal test—prior to exposure.
RB-1 = rose bengal test—second day postexposure.
RB-2 = rose bengal test—seventh day postexposure.
RB-3 = rose bengal test—ninth day postexposure.

There is a significant delay in excretion time in animals receiving chloroform compared to those receiving Fluothane and compared to their own controls. There is no significant difference between the two chloroform groups or in the uptake time.

TABLE 3

SEVERITY OF HEPATOTOXIC INFLUENCES OF THE ANESTHETIC AGENTS EMPLOYED (MOST SEVERE TO LEAST SEVERE)

Dog Number	Anesthetic Agent
648	Chloroform analgesia
1039	Chloroform anesthesia
1715	Chloroform anesthesia
368	Chloroform analgesia
370	Chloroform anesthesia
349	Chloroform analgesia
1990	Chloroform anesthesia
613	Chloroform anesthesia
635	Chloroform analgesia
637	Chloroform analgesia
1046	Fluothane anesthesia
605	Fluothane anesthesia
334	Fluothane anesthesia
5	Ether anesthesia
87	Ether anesthesia
89	Ether anesthesia

three and among the last six animals listed (table 3).

For each of these nine animals, the rose bengal control T_u value for that animal was subtracted from the sum of the T_u values for RB-1, RB-2 and RB-3 and the nine animals were ranked in order of greatest to least differences. This was done also for T_e values. The ranking of severity of histopathologic changes for each of these animals as outlined previously was utilized for correlation purposes. Results are shown in table 4. Statistically significant correlation could not be achieved between histopathologic evaluation and rose bengal results for these nine animals.

DISCUSSION

The objective of this work was an evaluation of the effects on the liver of certain anesthetic agents. This objective could not be realized by statistical analysis under the conditions of the experiment. A review of the conditions of the experiment is necessary in order to delineate sources of error. Even though the protocol was adhered to it was obvious that the dictates of good experimental design were not always satisfied.

Perhaps the primary deficiency was the lack of consistently controlled procedure in the rose bengal tests. This deficiency influenced the statistical analyses since all analyses included rose bengal test results. The two major difficulties in the radioactive procedures were those of (1) positioning and (2) "background" radiation fluctuations. Positioning of the scintillation counter and animal with respect to each other appeared to be of critical importance and small errors in the tracings would be greatly amplified during the computational manipulations necessary for deriving T_u and T_e values. Occasionally unexpected fluctuations in "background" radiation due to extraneous influences, *i.e.*, a radioactive watch dial, posed problems while counting procedures were in progress.

Inability to obtain suitable tissue specimens despite repeated attempts occurred on several occasions. For the sake of uniformity in tissue processing and staining all specimens were stored in formalin pending the completion of all biopsies. This resulted in varying degrees of leaching of glycogen, lipid, and perhaps protein constituents from the tissues. Calculations of the actual concentrations of anesthetic agents delievered during chloroform "analgesia" and chloroform anesthesia disclosed considerable overlapping. The histopathologic observations and radioactive rose bengal results referable to the four postanesthetic deaths (three due to chloroform anesthesia and one due to chloroform "analgesia") were not used in statistical analysis although the biological implications were of obvious significance.

An expectation of excellent correlation between liver structure and function is probably unreasonable. The limitations of the radioactive rose bengal method as a single test of liver function in experimentally induced hepatic injury are not known. Greater refinements of histologic methods, cytochemical and histochemical technics would undoubtedly facilitate precision with respect to correlations between liver function test and microscopic observations.

Certain information of value was gained from this study. The control observations yielded structural and functional test data which could be correlated statistically. This leads to the conclusion that the methods employed for control studies prior to anesthesia were essentially sound and induced no adventitious effects on normal liver structure and function. Chloroform in "analgesic" or anesthetic concentrations produced considerable structural changes in the liver whereas Fluothane and ether produced only minimal alterations. This appears to be substantiated by the consistency found in the histopathologic evalua-

tions. During the administration of the anesthetics there were no instances of hypotension which might have effected the results of these liver function studies.

The studies on the morphologic changes produced by chloroform yielded data which differed from published observations on chloroform hepatotoxicity. The degree of variability of injury, the preponderance of necrobiotic rather than autolytic cellular changes, the relative modest fatty changes, and the relatively slow initial degenerative and regenerative responses were notable.

TABLE 4

RANK CORRELATION: HISTOPATHOLOGY AND RADIOACTIVE ROSE BENGAL TEST

Animal Number	Agent	Histopathology Rank	T_u Difference Rank	T_e Difference Rank
368	Chloroform analgesia	1	3	2
370	Chloroform anesthesia	2	7	1
349	Chloroform analgesia	3	1	4
1990	Chloroform anesthesia	4	8	5
613	Chloroform anesthesia	5	9	3
635	Chloroform analgesia	6	2	6
1046	Fluothane anesthesia	7	4	7
334	Fluothane anesthesia	8.5	5.5	8.5
605	Fluothane anesthesia	8.5	5.5	8.5

T_u = uptake halftime, T_e = excretion halftime.
Nine animals with complete series of biopsy specimens and rose bengal tests were ranked from 1 through 9 as greatest to least change in histologic appearance and deviation from control from rose bengal tests. There was no significant correlation between histologic appearance of the biopsy section and function of the intact liver as measured by the rose bengal test.

SUMMARY

Dogs were subjected to chloroform anesthesia, or analgesia, or Fluothane or ether anesthesia. They were observed for nine days with serial liver biopsies and radioactive rose bengal uptake-excretion tests as measures of liver structural and functional changes. There was no statistical correlation between liver structure and function as measured following any of the anesthetics.

Chloroform in either anesthetic or analgesic concentrations produced hepatic changes in both structure and function which were evident by the second day and were generally more severe than those produced by Fluothane or ether. However, in most cases, these changes had returned to normal by the ninth day. Structural changes showed necrobiosis rather than necrosis of the coagulative type. The four deaths in the series followed chloroform administration.

Positioning of the scintillation detector and fluctuations in "background" radiation created difficulties in interpretation of the rose bengal test results. However, we believe that this test was reliable as a single test of liver function. The fact that control observations yielded results that correlated statistically suggests that the methods of testing were sound and could be used in other studies of this type.

The Fluothane was supplied by Ayrest Laboratories, Inc., New York, New York. The Tecota inhaler was loaned by Canam Co., Toronto, Canada.

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