FLUOTHANE TOXICITY: PATHOLOGICAL STUDIES OF MOUSE LIVER AND KIDNEY

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IT HAS been known for a number of years that halogenated hydrocarbons are capable of producing serious liver damage. Other factors such as oxygen lack or inadequate dietary intake can produce fatty degeneration. Not infrequently it is the combined effect of these factors which brings about marked pathological changes (1).

During the early phases of investigation on the halogenated hydrocarbon, Fluothane, J. Raventós (2) did some liver and kidney toxicity studies. The pathological report in his study said, in part: "The most consistent histopathological change seen in animals [rats, dogs, and monkeys] treated with Fluothane occurs in the kidney. It consists of dilatation of the proximal convoluted tubules and is associated with slight cytological change in the cells of these tubules. There are also minimal changes in the liver. These are of trivial extent and degree compared with those seen in a monkey anaesthetized on one occasion with chloroform or with changes known to occur in man after chloroform anaesthesia."

Furthermore, Raventós reported, "Microscopic examination of the livers of animals anaesthetized for long periods of time, or repeatedly, with Fluothane showed only minor change, a result that has been corroborated by biochemical tests. In no animal was fatty degeneration of the liver found, a frequent result of chloroform and divinyl ether anaesthesia."

In quest of more information on this subject, a study of the pathological status of mouse liver and kidney after exposure to anaesthetic doses of Fluothane was undertaken. Details of this investigation are the substance of this report

METHODS OF INVESTIGATION

The experimental mice were anaesthetized in a 1 to 2 per cent Fluothane vapour. To produce adequate anaesthetic conditions a modified Kochmann (1912) apparatus was utilized This equipment consists basically of: a syringe loaded with liquid Fluothane capable of being emptied at a constant rate, gaseous oxygen capable of being released at a relatively constant flow, a vaporizing chamber kept at a temperature slightly above the boiling point of Fluothane, a mixture chamber for O_2 and Fluothane vapour, an anaesthetizing chamber for small animals, equipped with a CO_2 absorber. This apparatus permits the administration of a controlled concentration of Fluothane-oxygen to small animals.

In all experimental animals (Tables I to IV) sections of liver and kidney were prepared as follows: (*a*) fixed in formol-alcohol, (*b*) impregnated with paraffin, and (*c*) treated with haemotoxylin and eosin stain. The livers of ten mice (Table V) were also examined by a standard fat-staining technique, that is, the tissue was (*a*) fixed in formol-saline, (*b*) quick frozen, and (*c*) stained with Sudan III

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Standardization

Male, Fwiff albino mice of approximately 27 gm. were used throughout the experiment. They had constant pre- and post-anaesthetic care. A standard diet of Buckerfield's animal food was provided. It was noted that the eating habits of the experimental mice were not grossly altered by exposure to the anaesthetic agent

The anaesthetic technique used was similar for all groups. The animals were exposed to anaesthetic doses of Fluothane vapour (approximately 1½ per cent) for a specified time.

Pathological examination was done by a single pathologist (Dr. Harold E. Taylor). The slides were all fixed and stained in a constant manner by the same technician. A double blind technique was utilized in presenting the slides for reading.

Experiment

Group 1. Thirty mice were anaesthetized for 45 minutes: (*a*) ten of this group were killed two days post-anaesthesia; (*b*) ten were killed five days post-anaesthesia, (*c*) ten were killed ten days post-anaesthesia (see Table I)

TABLE I

Results of Pathological Examination of Liver Tissue after 45-minute Exposure to Anaesthetic Haemotoxylin and Eosin Stain

	Fatty degeneration	Cellular change	Normal	Total number examined
Killed 2 days post-anaesthesia	7	1	2	10
Killed 5 days post-anaesthesia	0	3	7	10
Killed 10 days post-anaesthesia	0	7	3	10

Group 2. A control group of ten unanaesthetized mice was killed after having lived ten days under standard conditions (see Table II).

TABLE II

Results of Pathological Examination of Liver Tissue in Control Group (No Exposure to Anaesthetic) Haemotoxylin and Eosin Stain

	Fatty degeneration	Cellular change	Normal	Total number examined
Killed after 10 days in the animal house under standard conditions	0	1	9	10

Group 3. Thirty mice were anaesthetized for 45 minutes on five consecutive days; total time of exposure to anaesthesia was 3 hours, 45 minutes: (*a*) ten of this group were killed two days after completion of the serial exposure; (*b*) ten were killed six days after completion of the serial exposure; (*c*) ten were killed ten days after completion of the serial exposure (see Table III)

TABLE III

RESULTS OF PATHOLOGICAL EXAMINATION OF LIVER TISSUE AFTER 45-MINUTE EXPOSURE TO ANAESTHETIC ON 5 CONSECUTIVE DAYS HAEMOTOXYLIN AND EOSIN STAIN

	Fatty degeneration	Cellular change	Normal	Total number examined
Killed 2 days post-anaesthesia	6	2	2	10
Killed 6 days post-anaesthesia	1	2	7	10
Killed 10 days post-anaesthesia	0	2	8	10

Group 4. A repeat group of ten mice, anaesthetized for 45 minutes on five consecutive days, then killed two days post-anaesthesia, was done This time fat stains were done on liver tissue in addition to the usual haemotoxylin and eosin stains (see Tables IV and V).

TABLE IV

RESULTS OF PATHOLOGICAL EXAMINATION OF LIVER TISSUE AFTER 45-MINUTE EXPOSURE TO ANAESTHETIC ON 5 CONSECUTIVE DAYS HAEMOTOXYLIN AND EOSIN STAIN

	Fatty degeneration	Cellular change	Normal	Total number examined
Killed 2 days post-anaesthesia	9	1	0	10

TABLE V

RESULTS OF PATHOLOGICAL EXAMINATION OF LIVER TISSUE AFTER 45-MINUTE EXPOSURE TO ANAESTHETIC ON 5 CONSECUTIVE DAYS· SUDAN III FAT STAIN

Mouse	Results (Total involvement of lobule recorded as +4)
1	+2
2	+3
3	±
4	+1
5	+2
6	+3
7	+3
8	+3
9	+2
10	+2

Results

In the kidney studies no gross or microscopic alteration in renal structure was seen.

In the liver studies no specific alterations were found on gross examination. Microscopically three changes were noted: (i) vacuolar formation—fatty change, (ii) nuclear change—excessive numbers of binucleated cells, excessive numbers of large nucleated cells, pyknosis of nuclei excessive; (iii) zonal areas of cloudy swelling with pallor of cells.

From Tables I to IV, it will be noted that a high percentage of livers examined two days after exposure to anaesthesia showed evidence of fatty degeneration, twenty-two out of thirty. Tissue examined at longer intervals post-anaesthesia frequently showed some nuclear changes. By this time, vacuoles were no longer discernible. The fatty change produced by Fluothane in mouse liver is the usual transient type.

In group 4 (mice exposed to 45 minutes of anaesthesia on five consecutive days and then killed two days later), the fatty change observed via haemotoxylin and eosin slides correlated 100 per cent with that seen on slides prepared with special fat stains (see Tables IV and V) It will be noted that nine out of ten in this group showed fatty change The degree of fatty change is recorded. It is graded with total involvement of a lobule indicated by +4. For details, see Table V.

Summary

A study of the pathological status of mouse liver and kidney after exposure to anaesthetic doses of Fluothane was done The findings as recorded indicate a transient fatty change in mouse liver. This was demonstrated by vacuole formation seen on haemotoxylin and eosin slides when the experimental animal was killed two days post-anaesthesia. In the re-check series (Group 4), 100 per cent correlation between tissue stained with haemotoxylin and eosin and special frozen-section fat-stained slides was found.

No gross or microscopic alterations in kidney structure were seen

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RÉSUMÉ

Nous avons fait une étude pour nous rendre compte de l'existence de pathologie dans le foie et les reins de souris soumises à des doses anesthésiques de Fluothane. Nous avons employé la souris comme animal de laboratoire. L'appareillage anesthésique employé était un appareil Kochmann (1912) modifié.

Nous n'avons constaté aucune modification macroscopique ou microscopique dans la structure rénale. Chez un grand nombre de souris soumises au Fluothane, nous avons constaté une franche dégénérescence graisseuse du tissu hépatique aux environs de la veine centrale. Nous avons pu faire ces constatations sur des coupes venant d'animaux tués deux jours après l'anesthésie. Lorsque les coupes de tissu venaient d'animaux tués cinq à dix jours après l'anesthésie, nous n'avons pas pu observer de formations vacuolaires. Cependant, à ce moment-là, nous avons noté sur certaines coupes des changements nucléaires, i.e., de multiples gros noyaux et de multiples cellules binucléées.

Dans les groupes de vérification (Tables IV et V), les résultats sur les coupes hemotoxylin et eosin correspondaient 100% à des préparations spéciales de trainées de graisse gelée.

REFERENCES

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