

Trifluoroacetic Acid and Some Possible Intermediate Metabolites of Halothane as Haptens

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Methods

To investigate whether metabolites of halothane could act as haptens and thus account for part of the hepatic damage sometimes seen after repeated halothane anesthetics, known and suggested metabolites of halothane were coupled to chicken serum globulin. Rabbits were subsequently immunized with these complexes, and the production of antibodies was tested by immunodiffusion in agar gel. In all cases similar precipitin lines were formed against the complexes used for immunization in each rabbit, as well as against the trifluoro-compound coupled to the rabbits' own serum proteins, bovine serum albumin, or poly-L-lysine. This indicates that the trifluoro-compounds studied form partly identical antigenic determinants and can act as haptens. (Key words: Halothane; Haptens; Biotransformation; Fluoride.)

THE CAUSE of the hepatic necrosis sometimes seen after halothane anesthesia has recently been attributed to a possible hapten function of the metabolites of halothane.¹⁻⁵ Trifluoroacetic acid, the main metabolite in urine,^{6,7} and possible intermediate metabolites, trifluoroethanol and trifluoroacetaldehyde,⁸ cause fatty changes in mouse liver.⁹ Clinical evidence,¹⁰⁻¹² together with the demonstration of halothane-induced lymphocyte stimulation² and the occurrence of antimitchondrial antibodies¹³ in patients jaundiced after exposure to halothane, suggests, however, that hypersensitivity rather than direct hepatotoxicity is the cause of the hepatic damage. Therefore, we studied trifluoroacetic acid and possible intermediate metabolites of halothane to find out whether they can act as haptens.

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Complexes of chicken serum globulin and trifluoroethanol (Fluka AG, Buchs SG), trifluoroacetaldehyde hydrate (Pierce Chem. Co., Rockford, Illinois), trifluoroacetic acid (Fluka AG, Buchs SG), and trifluoroacetic anhydride (Fluka AG, Buchs SG) were prepared by mixing an aqueous solution of the protein (10-15 mg/ml) and the trifluoro-compound (1.2 M) in a ratio of 1 to 1.5 at 4 C for 15 minutes. This was followed by dialysis against distilled water overnight, to remove the excess of the trifluoro-compound, and lyophilization. Three mg of each complex dissolved in 0.5 ml of distilled water were injected, together with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan), subcutaneously into rabbits once a week for ten months. Two rabbits were used for each of the complexes with trifluoroethanol, trifluoroacetaldehyde hydrate, and trifluoroacetic anhydride, and three rabbits were used for the complex with trifluoroacetic acid. Serum samples were taken from all rabbits prior to the immunization and after three, six, and ten months by bleeding of the ear veins.

The immunologic properties of the serum samples were investigated by the microimmunodiffusion method, described by Linder,¹⁴ in 1 per cent agar gel in phosphate-buffered saline solution, against similarly prepared complexes of the rabbits' own serum proteins, bovine serum albumin, and poly-L-lysine (molecular wt 3-5 × 10⁵).

Results

All immune sera reacted against all of the different antigens independently of the antigenic complex used when immunization was continued for ten months (fig. 1a, fig. 2). No immunologic precipitation could be demon-

† Trifluoroacetic anhydride is not a suggested intermediate metabolite of halothane, but was included in the study because of its reactivity.

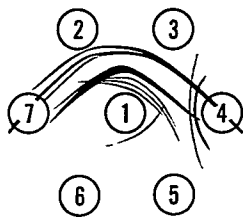
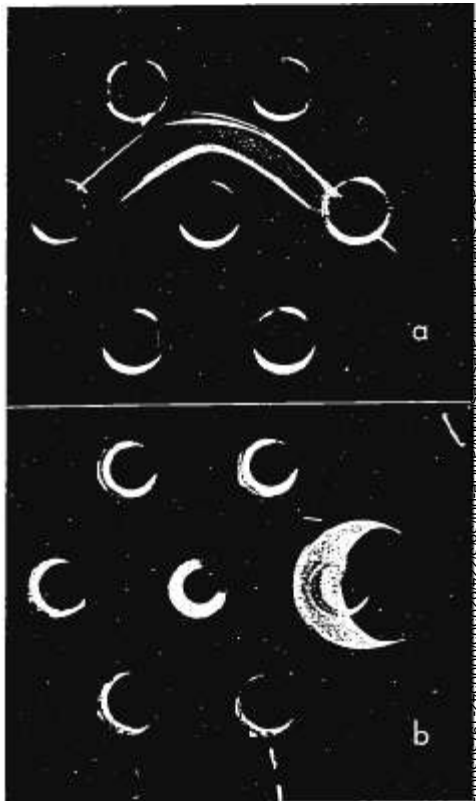


FIG. 1. *a*, agar plate, showing the immune serum (1) from a rabbit immunized for ten months with trifluoroacetic acid-chicken serum globulin tested by immunodiffusion against: chicken serum globulin (2); trifluoroacetic acid-chicken serum globulin (3); trifluoroacetic acid-poly-L-lysine (4); trifluoroacetic acid-rabbit serum protein (5); rabbit serum protein (6); poly-L-lysine (7). The precipitin lines in the agar gel, also depicted in the schematic figure, indicate antigen-antibody reactions. The diffuse precipitation around trifluoroacetic acid-poly-L-lysine (4) is not an immunologic reaction.

b, agar plate, showing the serum collected prior to the immunization of a rabbit tested against some of the antigenic complexes. The nonimmune serum is in the middle and the antigenic complexes are located as in *a*. Only non-immune diffuse precipitation in the vicinity of trifluoroacetic acid-poly-L-lysine (4) can be seen.



strated when the antigenic complexes were tested against the nonimmune sera collected prior to immunization (fig. 1*b*). After three months of immunization no specific reaction against the trifluoro- compound complexes was seen with any one of the sera. In six months the sera from the animals injected with the trifluoroacetic anhydride complex gave strong, distinct precipitin lines in the agar gel, but trifluoroacetic acid-complex antisera and trifluoroacetaldehyde hydrate-complex antisera reacted also, weakly, against the antigens, and the precipitin lines were clearly distinguish-

able from those produced against the carrier protein alone. Not until immunization had been continued for ten months were there weak precipitin lines when the antigens were tested against the trifluoroethanol-complex antisera. In that time the potencies of the antisera from the rabbits immunized with the trifluoroacetic acid complex and trifluoroacetaldehyde hydrate complex had increased, and seemed to be of the same order as that of the trifluoroacetic anhydride-complex antisera. No attempt was made to quantitate the reactions exactly.

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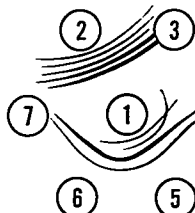
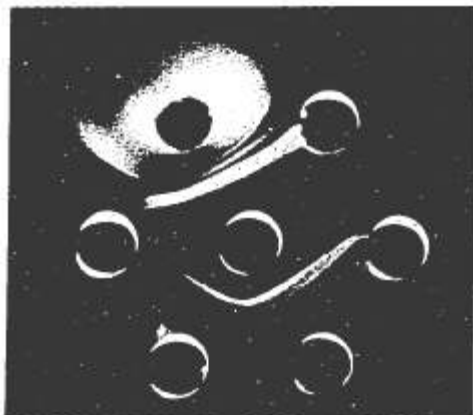


Fig. 2. Agar plate, showing the immune serum (1) from a rabbit immunized for ten months with trifluoroacetaldehyde hydrate-chicken serum globulin tested by immunodiffusion against: trifluoroacetaldehyde hydrate-poly-L-lysine (2); poly-L-lysine (3); trifluoroacetaldehyde hydrate-bovine serum albumin (4); trifluoroacetaldehyde hydrate-chicken serum globulin (5); chicken serum globulin (6); bovine serum albumin (7). The most important precipitin lines are depicted in the schematic figure.

The immune sera also reacted with complexes of the trifluoro- compounds and rabbit serum proteins, bovine serum albumin, and poly-L-lysine. Thus, great specificity for the trifluoro- component was revealed, as the antisera reacted with protein complexes phylogenetically completely different from those used for immunization.

Discussion

Molecules the size of halothane are generally not known to act as haptens in the sense that specific antibody formation would be stimulated by them alone.¹⁵ Unidentified complexes of trifluoro- groups and macromolecules, however, have been recognized autoradiographically and radiochromatographically in laboratory animals after halothane anesthetics,^{4, 16} and trifluoroacetyethanolamine has been detected in the urine of one patient.³

Since halothane is mainly metabolized in the liver, it is possible that the metabolites form complexes with liver-specific proteins, resembling those used in the present animal study, and thus, immunologic sensitization against such complexes would be elicited.

That the different sera cross-reacted similarly with the various antigens provides evi-

dence for the concept that in the preparation of the antigenic complexes the trifluoro- groups remained unaffected and, therefore, probably served as part of the antigenic determinant. Because the antisera also reacted with each trifluoro- compound-poly-L-lysine, it is possible that another part of the antigenic determinant consists of one molecule or a sequence of molecules of L-lysine.

The bond between the C-1 carbon of the trifluoro- compounds and poly-L-lysine or the proteins was not analyzed, but the identification of trifluoroacetyethanolamine by Cohen,³ together with our own experience in toxicologic studies of metabolites of trifluoro-hydrocarbons,¹⁷ indicates binding to $-\text{NH}_2$ and $-\text{SH}$ groups.

The metabolic pathway from halothane to trifluoroacetic acid is unsettled. In spite of extensive studies, no organic metabolite other than trifluoroacetic acid has been identified.^{3, 4, 18, 19} Were it not for intracellularly formed trifluoroacetate,^{19, 20} the reactive trifluoroacetaldehyde^{5, 17} would be able to form complexes with proteins *in vivo* after halothane anesthesia; however, the relationship of the present data to clinical conditions remains to be demonstrated.

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Paraphernalia

THERMODILUTION CARDIAC OUTPUT The Swan-Ganz catheter is a balloon-tipped double-lumen tube which can be passed from a peripheral vein through the right heart and into the pulmonary artery. Through it can be measured right atrial, pulmonary arterial, and pulmonary wedge pressures. This catheter has now been modified by adding a thermistor near the tip, thus permitting measurement of cardiac output by the thermodilution technique. Fidelity of pressure response through the catheter, and accuracy and precision of the thermodilution method, were measured and found satisfactory. This catheter was used in 33 patients with no apparent complications. Repeated right and left ventricular function curves were made, which permitted better assessment of therapy. (Forrester, J. S., and others: *Thermodilution Cardiac Output Determination with a Single Flow-directed Catheter*, Amer. Heart J. 83: 1972.)