Some Properties of Fluorocarbon-Treated Animal Tissue.* (25398)

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This report is concerned with changes occurring in tissue subjected to extraction with fluorocarbon, trifluorotrichloroethane, CCl_2F-$CClF_2$. Although this type of solvent is finding increasing use in separation of viruses and viral constituents from host tissue, relatively little is otherwise known about the constitution of such extracts, and the changes they undergo at successive extraction stages. Some of our results in exploring the possibility of applying the procedure to aid in detecting and concentrating mouse tumor agents will also be indicated.

Methods. A 25% homogenate of tissue in 0.04 molar versene at pH 7 was homogenized for 5 minutes at high speed in a VirTis "23" apparatus with ½ its volume of trifluorotrichloroethane in the cold. After centrifugation at 2000 × g for 5 minutes, the turbid aqueous phase was removed and again homogenized with ½ its volume of solvent. The process was repeated for 5 or more extraction cycles. Protein, RNA and DNA were determined on the aqueous phase at each stage. An aliquot was centrifuged at 120,000 × g for 1 hr, and these constituents again determined on the supernatant to obtain sedimentable components. The final aqueous phase was concentrated by dialysis against polyvinylpyrollidone, then against 0.05 M phosphate, pH 7, and examined in the analytical ultracentrifuge at 196,000 x g at a 1% protein concentration. RNA was determined by the orcinol method, DNA by the indole reaction, and protein by the biuret procedure, using appropriate standard curves.

Results. As extraction proceeded, the homogenate was gradually depleted of its protein, RNA and DNA, the amount removed varying with the constituent and the preparation. An illustration is given in Fig. 1. In this case, acid insoluble RNA had already disappeared by the fourth extraction stage, whereas appreciable amounts of protein and DNA remained to the end. In general, protein was the one constituent which withstood removal by the solvent even after 7 extrac-

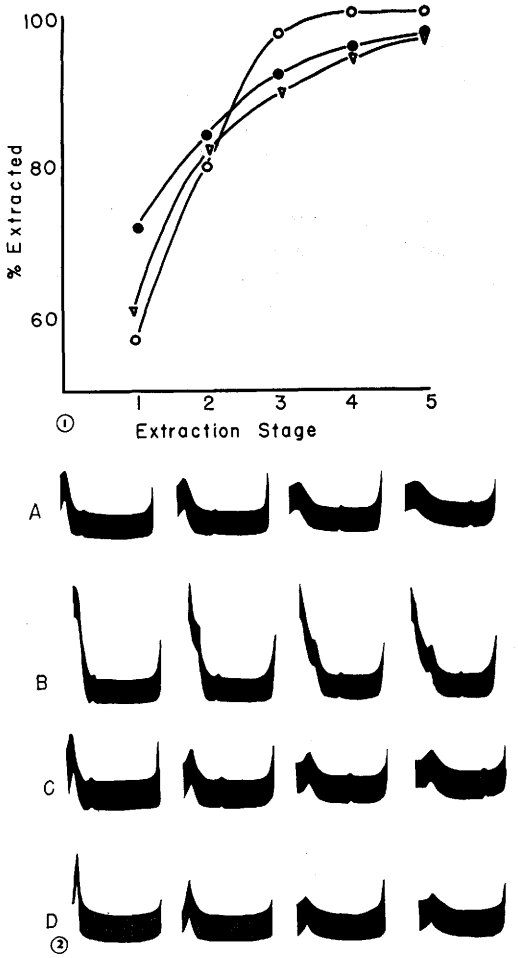

FIG. 1. Total RNA, DNA and protein in aqueous phases of fluorocarbon extracts of C3H mammary carcinoma at different extraction stages. ○ RNA, ● DNA, △ protein.

FIG. 2. Sedimentation curves of stage 5 fluorocarbon extracts in .05 M phosphate, pH 7. A, B, C and D are as indicated in Table II. Photographic exposures were at 8 min. intervals after a gravitational force of 196,000 × g was reached in a Spinco Model E centrifuge. Sedimentation constants of A, B and C were 23.4, 38.1 and 37.2, respectively.

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TABLE I. Sedimentable* Components in Fluorocarbon Extracts of the Mouse Mammary Tumor at Successive Extraction Stages.

Component	% sedimented at extraction stage 0	1	2	3	4	5
Protein	55	32	19	19	10	9
RNA	81	68	27	0		
DNA	96	91	87	87	78	70

* 120,000 × g for 1 hr.

tions. The terminal protein may be a fairly pure component. Thus, 67% of the protein of the Ehrlich ascites cells remaining after 5 extractions migrated as a single peak at pH 8.8 in the Tiselius apparatus.

Protein, RNA and DNA existed in both sedimentable and non-sedimentable forms in early extraction stages, whereas in advanced stages, one or more of the components were entirely unsedimentable, as for example, RNA at stage 3 of Table I. The RNA remaining at this stage was completely degraded, as determined by its solubility in cold 0.25% uranic acetate in 2.5% perchloric acid, thus accounting for its behavior. However, this was not the sole explanation for part of the RNA being non-sedimentable in earlier extraction stages, as in stage 2, where 73% of the RNA was non-sedimentable, but only 58% acid soluble. None of the non-sedimenting protein or DNA was acid soluble.

Fluorocarbon extracts of 3 mouse tumors and of normal mouse kidney were concentrated and centrifuged at 120,000 × g for one hour. The results are shown in Table II. The differences in sedimentable RNA and DNA of these tissues are noteworthy. In the analytical centrifuge, a rapidly sedimenting component, representing about 5% of the total material, was observed in all 3 tumor preparations (Fig. 2), but absent from the normal mouse kidney. Similar preparations, derived from mixed viscera of normal mice of various strains, of their individual organs, and of whole fetuses, also failed to exhibit a sedimenting component of this type.

Discussion. Appearance of a unique particulate component in the 3 tumors, which was absent from normal tissues, is of particular interest. This substance was not observed in extracts before fluorocarbon treatment, possibly because it was obscured by normal tissue constituents. It is tempting to consider that this material may be of viral origin inasmuch as all 3 tumors probably have a viral component in their etiology. This is, of course, well established for the mammary carcinoma; the PL leukemia is in all respects analogous to the better known AK leukemia, and is therefore likely to be a viral neoplasm; the thymic tumor arose in a 9 month old C3H mouse that had been inoculated *i.v.* at 2 mos. with a cell-free PL leukemic extract, and would also therefore appear to be a viral neoplasm. This possibility is strengthened by the observation (unpublished) that fluorocarbon extracts of transplants of this tumor exhibit virus-like particles having an average diameter of 120 mμ.

The low sedimentation constants of these components (Fig. 2) suggest that they are not complete viruses. For example, the mammary tumor agent is reported to be several hundred S units (1), but the corresponding component we observed is only 38.1 units. Conceivably, the estimated 95% impurity could have strongly influenced the observed value. An alternative explanation is that the component was an incomplete virus (2).

It must be pointed out that the analytical data for RNA and DNA (Table II) do not clearly distinguish normal tissue from tumor tissue. But certain inferences may nevertheless be drawn. Thus, if the mammary agent is

TABLE II. Sedimentable* Components of Concentrated Stage 5 Fluorocarbon Extracts.

Exp.	Tissue	Conc. factor	Component	Amt sedimented μg/ml	Amt sedimented %
A	C3H thymic tumor	16	Protein	5000	23
			RNA	210	4
			DNA	0	0
B	C3H mammary tumor	3.4	Protein	2600	30
			RNA	0	0
			DNA	70	53
C	PL leukemic lymph nodes	2.7	Protein	1100	22
			RNA	55	7.2
			DNA	60	5.6
D	Normal mouse kidney	11.7	Protein	1320	13.9
			RNA	.8	1
			DNA	16	18.9

* 120,000 × g for 1 hr.
† From an inbred Swiss (Tumblebrook) strain.

resistant to extraction by the fluorocarbon, as appears to be the case(3), then it is more likely to be a DNA than RNA virus, for there was no intact RNA in this particular preparation. Similarly, the fluorocarbon extract of the thymic tumor had no DNA, hence was more likely to be a RNA virus. The leukemic tissue had both RNA and DNA but by inference, only the RNA would be expected to contain a viral component.

Summary. RNA, DNA and protein are differentially removed by extraction of a tissue with fluorocarbon CCl_2F-$CClF_2$. These constituents occur in sedimentable (120,000 x g, 1 hour) and non-sedimentable forms. A unique component was observed in concentrated fluorocarbon extracts of 3 neoplastic tissues, which was absent from normal tissue. Its possible relation to viruses is discussed.

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Distribution Studies on Radioactive Hg^{203} Sodium Mercaptomerin in Animals. (25399)

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Many previous studies on mercurial diuretics have been performed on compounds administered clinically orally(1,2), or parenterally when combined with theophylline(3). This investigation was undertaken with Hg^{203} mercaptomerin sodium because, as employed clinically, it is not combined with any agent which might influence distribution or excretion. This study was done to determine general distribution, renal concentration, and rate of urinary excretion of this compound.

Materials and methods. Hg^{203} mercaptomerin sodium was synthesized in our laboratories,* and was identified by paper chromatography, quantitative determination of Hg and S, determination of temperature of decomposition, and determination of diuretic activity. The solutions were freshly prepared with pyrogen-free water and resterilized by filtering through type H. A. millipore filter. Specific activity of original material was 360 μc/mg mercury when assayed for gamma radiation. A standard dose of 1 mg Hg kg (equivalent to 3.5 mg kg mercaptomerin sodium) was used. Thirty-three albino rats (165-190 g), injected intraperitoneally, were divided into groups of 3 and placed in metal metabolism cages. Each group was deeply anesthetized with ether and sacrificed 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, or 300 minutes after injection of the radioactive compound. Blood was obtained by cardiac puncture, using heparin as anticoagulant, and both kidneys were removed, decapsulated, weighed, and digested in concentrated nitric acid. Average radioactivity of renal tissue is expressed as μg of mercaptomerin sodium/g wet weight of tissue. Aliquots from pooled urines of 60, 120, 130, and 240 minute groups were collected and assayed for radioactivity. In addition, a series of kidney sections of the 1, 2, 3, and 4 hour groups was prepared for radioautographs. Sections 8 μ thick of kidney tissue were prepared by usual paraffin impregnation technic and mounted without removal of paraffin. The slides were covered with Kodak Panchromatic XX photographic film and firmly fastened with tape. Optimum exposure time was 14 days. In a second study, Hg^{203} mercaptomerin sodium was injected in-

* Method of synthesis supplied by Wyeth Inst. for Medical Research, Radnor, Pa.