

50% of the radioactivity was recovered from this organ. The distribution of radioactivity in the cow indicated that the polymer was preferably accumulated in organs containing reticuloendothelial cells.

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Effect of Nonionic Detergents on Electrophoretic Distribution of I-131-Labeled-Thyroid Hormones in Serum.* (29591)

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During the course of studies concerned with the effects of nonionic surface-active agents on the electrophoretic properties of serum proteins, striking changes were noted in the migration patterns of I-131-labeled thyroid hormones. Consequently, a series of experiments was carried out in which varying concentrations of nonionic detergents were examined for their abilities to alter the electrophoretic partition of thyroxine-I-131 and triiodothyronine-I-131 among serum proteins.

Materials and methods. Either Triton X-100[†] (a nonionic alkylphenoxy polyethoxy ethanol) or Tween 80 (polysorbate 80) was added in varying concentrations to 0.5 ml aliquots of normal serum. These were followed by addition of 0.01 ml of a 1% solution of human serum albumin containing 1.0 μ c of I-131-labeled-l-thyroxine or -l-triiodothyronine[‡] after which the entire mixture was stirred vigorously with a wooden rod.

Triton X-100 was used without further dilution, but because of its viscosity, it was necessary to dilute the Tween 80, 1:3 with distilled water.

Zone electrophoresis was carried out on strips of Whatman No. 3 filter paper (1¼ × 18½ inches) folded in half and suspended vertically over a glass rod in a closed lucite cell. The free ends of the paper extended 1¼ inches into baffled compartments containing platinum wire electrodes and barbital buffer (pH 8.6, ionic strength 0.075).

Ten to 20 microliters of the serum mixture were applied in a narrow band along the apex of the paper and covered with the buffer solution (the paper had been previously saturated with barbital buffer except for the region at the apex). An electric potential of 90 to 110 volts (6 to 9 ma) was applied for approximately 18 hours at room temperature, after which the paper strips were removed, dried in air and the radioactivity on the paper was located by means of a strip scanner connected to a continuous recorder or by autoradiography using X-ray film.

The proteins on the paper were stained

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[†] Rohm and Haas, Co., Phila., Pa.

[‡] Abbott Laboratories, Oak Ridge, specific activity, 25-40 mc/mg.

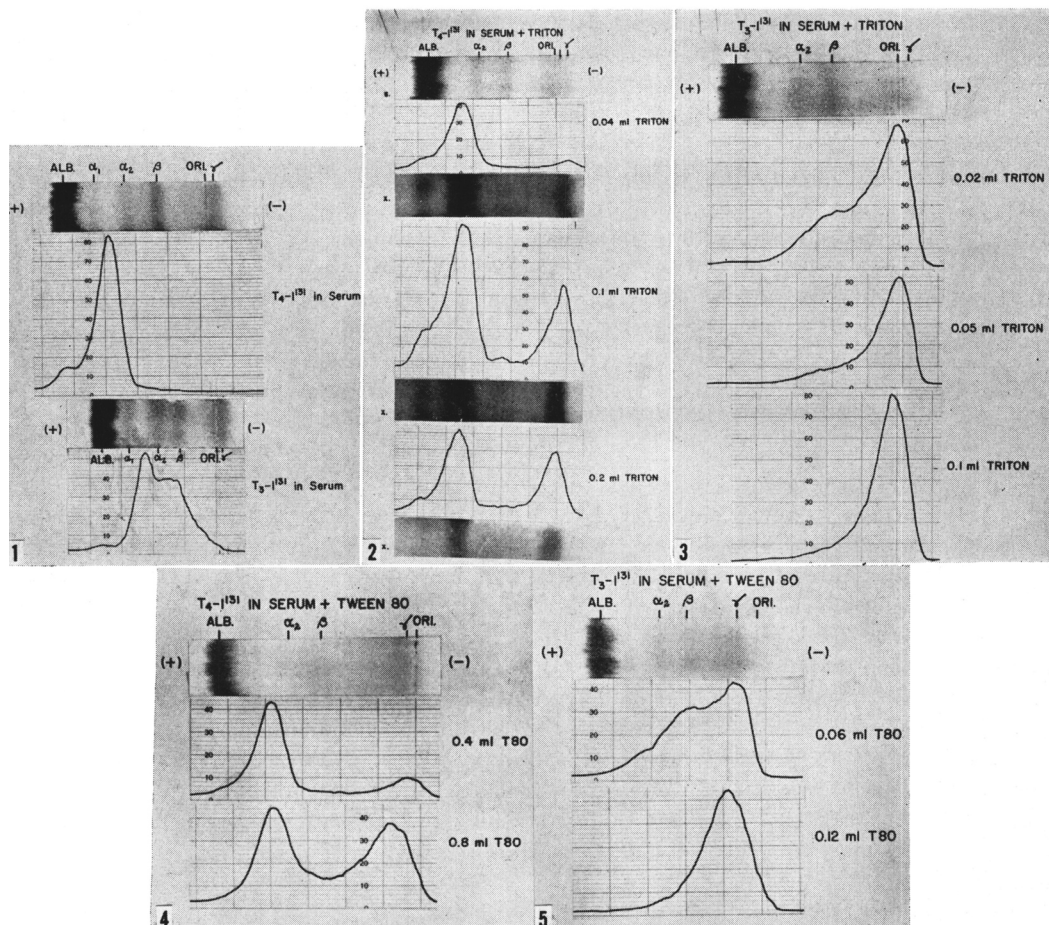


FIG. 1. Paper electrophoresis—conventional pattern of distribution of T₄-I-131 and T₃-I-131 in serum. Stained filter paper strip shown above recorded radioactivity.

FIG. 2. Paper electrophoresis of thyroxine-I-131 obtained with Triton X-100 added to serum. Autoradiographs (x) are shown beneath the recorded patterns of radioactivity.

FIG. 3. Electrophoretic patterns of Triiodothyronine-I-131 obtained with Triton X-100 added to serum.

FIG. 4. Pattern of Thyroxine-I-131 distribution using Tween 80 in serum.

FIG. 5. Pattern of Triiodothyronine-I-131 distribution with Tween 80 added to serum.

by soaking the strips for 15 minutes in 0.2% bromphenol blue in ethyl alcohol saturated with mercuric chloride and then rinsing the excess dye away with tap water. Since the detergents appeared to diminish the intensity of the stain on the protein fractions, the alpha 1 globulin could not always be identified.

Results and discussion. As shown in Fig. 1, thyroxine-I-131 (T₄-I-131) characteristically migrates with thyroxine-binding globulin (TBG), between the alpha 1 and alpha 2 globulins, whereas triiodothyronine-I-131 (T₃-I-131) usually moves with both the

inter-alpha and the beta globulins. The conventional pattern of distribution of T₄-I-131 and T₃-I-131 in serum, however, was abolished by the action of the nonionic surface-active agents. Addition of Triton X-100 resulted in the loss of the I-131-labeled thyroid hormones from the inter-alpha zone with the appearance of radioactivity in the zone of the gamma globulins (Fig. 2, 3). The magnitude of loss of T₄-I-131 from TBG to the gamma globulin region appeared to be related directly to the amount of surface-active agent used in the serum. Addition of Tween 80 (Fig. 4 and 5) resulted in similar changes

in distribution patterns, and just as with Triton X-100, the triiodothyronine-I-131 became dissociated from the conventional transport proteins but with substantially smaller concentrations of detergent.

Triton X-100 or Tween 80, when dissolved in saline containing I-131-labeled-thyroxine or triiodothyronine and then applied directly to filter paper exhibited peaks of radioactivity, after electrophoresis, which approximated the zone of the gamma globulins of control sera. Hence, it was not possible to ascertain whether the nonionic agents in combination with radio-hormones migrated freely, or migrated as a complex in association with the gamma globulins. Furthermore, the possibility that the detergents might have acted by increasing the affinity between the gamma globulin fraction and the thyroid hormones directly could not be excluded.

Consideration might be given to the possibility that denaturation of thyroxine-binding globulin by detergent action could have resulted in the impairment of transport by the inter-alpha globulin fraction with consequent adsorption of the "loose" thyroxine-I-131 at the origin of the filter paper. This does not seem likely, however, for had there not been active binding of radiothyroxine by the gamma globulin complex, the displaced thyroxine-I-131 should have migrated in association with albumin. Furthermore, if surface-active agents acted solely by damaging thyroxine-binding globulin and thereby im-

pairing the transport mechanism, the affinity between serum proteins and the labeled-hormones should be greatly reduced. The converse has been shown to obtain, however; plasma obtained from animals treated with detergents or plasma to which detergents were added had an augmented ability to bind triiodothyronine-I-131(1).

Conceivably, a similar mechanism of increased plasma binding with regard to cholesterol might be invoked to explain the hypercholesterolemia observed in animals shortly after administration of a nonionic surface active agent(2).

Summary. The conventional patterns of radioactivity observed after filter paper electrophoresis of serum containing I-131-labeled thyroxine or triiodothyronine were altered by addition of nonionic surface-active agents *in vitro*. Triton X-100 or Tween 80 added to serum interfered with the binding of T4-I-131 or T3-I-131 by TBG and resulted in the migration of radio-hormones in the zone of the gamma globulins. The concentration of radioactivity found in the region of the gamma globulins depended on the quantity of non-ionic detergent which had been added to the serum.

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Histamine Sensitivity in Mice of Different Ages after *Bordetella pertussis* Treatment or Adrenalectomy. (29592)

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The degree of histamine sensitization induced by *Bordetella pertussis* in mice is influenced by age(1,2,3). Thus, within a particular strain, young mice are less susceptible to this effect than older mice. For example, the LD₅₀ of histamine diphosphate for one

strain of 6-week-old *B. pertussis*-treated mice was found to be 54 mg/kg body weight, while it was only 17 mg/kg body weight for 12-week-old mice(1,4). The LD₅₀ for unsensitized adult mice of the same strain was greater than 1000 mg/kg body weight(4).