16*S* protein is completely converted to its 12*S* substituents after isolation and recentrifugation on the sucrose gradient. This is in sharp contrast to amino acid–labeled, lamb thyroglobulin which is stable under such conditions. It is not likely that the 16*S* and 12*S* proteins found in vivo are breakdown products of the 19*S* protein, since analysis of glands of rats labeled with $^{125}I$ before methimazole administration reveals that the remaining radioactivity is limited to the 19*S* region.

When thyroid slices from methimazole-treated rats are incubated with $^{125}I$, all species of soluble protein present in the slice are poorly but definitely iodinated (Fig. 3*B*). This presumably occurs as the methimazole preexisting in the slice is diluted during the incubation because administration of $^{125}I$ to such animals reveals virtually no iodination of the thyroid proteins. After discontinuation of methimazole there is a decrease in the 16*S* and 12*S* components with time, while the relative amount of the 19*S* protein increases (Fig. 4). Iodine labeling in vivo again follows the protein pattern and progressively increases with time after cessation of treatment with the drug. The specific activities of the iodine-labeled 16*S* and 12*S* proteins are somewhat lower than that of the 19*S* protein. Iodination of the 16*S* and 12*S* proteins can occur in vivo after release of the iodination block, but one cannot tell whether or not there has been a conversion of slowly sedimenting iodinated units to 19*S*.

Thus there is a block in the maturation of thyroglobulin both in thyroid slices from normal rats and in thyroids of rats treated with an agent which inhibits both conversion of iodide to organic iodo-compounds and thyronine formation. This block leads to an accumulation of labile, noniodinated, more slowly sedimenting, thyroglobulin-like molecules. Whether the defect in maturation of 16*S* molecules to 19*S* thyroglobulin can be attributed entirely to the lack of iodination or to block in coupling cannot be decided from these experiments. Possibly iodination prepares the molecule for other alterations which render the thyroglobulin more stable. In any case, our data emphasize the important role of iodination in the maturation of thyroglobulin and show that aggregation of thyroglobulin subunits to particles as large as 16*S* can proceed in the complete absence of iodination.

The 16*S* particle, formed by dimerization of 12*S* units, most likely undergoes conformational changes upon iodination to produce 19*S* particles. The 16*S* and 12*S* presumed thyroglobulin precursors can be iodinated in vivo. These conclusions are also supported by experiments on the association of isolated, labeled, thyroglobulin subunits (*12*), and by a recent report showing that the amino acid–labeled 17*S* component in sheep-thyroid slices is noniodinated (*13*).

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8. Soluble thyroid proteins were prepared for sucrose density-gradient (5 to 20 percent) centrifugation at 23,000 rev/min for 38 hours in the SW 25.1 rotor of the Spinco L2 ultracentrifuge (*2*). Optical density was recorded automatically with the Gilford Absorbance Recorder. Drops were collected in vials for radioactivity determination (*2*), except that a 0.1 ml portion of each gradient fraction was used in other procedures (see Figs. 1 and 3). Carbon-14-L-leucine (uniformly labeled, 1.7 mc/mg), L-leucine-4,5-$^{3}H$ (30 mc/mg), and carrier-free $^{125}I$ were obtained from New England Nuclear Corp. Male C-D strain rats (200 g) were obtained from the Charles River Laboratories.
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9 July 1965

## Fluorescent Contaminants from Plastic and Rubber Laboratory Equipment

Abstract. *Plastic and rubber laboratory apparatus contains materials that are extractable with water, aqueous solutions, and nonaqueous solvents. These extractable substances fluoresce under ultraviolet light and were found as contaminants in nutrient solution used for growing excised lemon fruit tissue in vitro. The source of these fluorescent contaminants has been removed by using all-glass laboratory equipment.*

In examining nutrient solutions for fluorescent compounds released from excised lemon fruit tissue growing in vitro (*1*), I have found fluorescent material not noted previously, in addition to the fluorescent substances usually present in nutrient solution containing proliferating tissue. This observation led to the finding that some fluorescent substances were contaminants, from plastic and rubber laboratory equipment, which had unknowingly been introduced during preparation of the nutrient solution and during chromatographic analysis of nutrient solution containing proliferating tissue. These contaminants stem from substances extracted from plastic and rubber apparatus by water, aqueous solutions, and nonaqueous solvents.

Primary sources of fluorescent contaminants were from: (i) polyethylene wash bottles containing glass-redistilled water; (ii) polyethylene reagent bottles containing dilute aqueous NaOH (0.1*N*); (iii) glass vessels, with black rubber stoppers, containing dilute aqueous NaOH (0.1*N*); (iv) Bakelite screw caps (with or without paper or rubber liners) on glass vials containing glass-redistilled water autoclaved for 20 minutes at 15 lb/in.$^2$ (1 atm) (*2*); (v) Bakelite screw caps (paper- or rubber-lined, or unlined) coming in contact with dilute aqueous NaOH; and (vi) black rubber stoppers, pure latex-type (pharmaceutical grade) rubber stoppers, and rubber liners for Bakelite screw caps coming in contact with alcohols (ethanol, methanol, 1-butanol, isopropanol, tertiary amyl alcohol) (*3, 4*). Fluorescence was detected by: (i) spotting the above-named liquids (except NaOH) that

made contact with plastic and rubber materials on chromatographic paper and examining the spots under ultraviolet light (3660 Å); and (ii) examining all the above-named liquids in quartz cuvettes under ultraviolet light (3660 Å). All the above-named solvents and solutions when stored in all-glass containers (controls), including glass-redistilled water autoclaved for 20 minutes at 15 lb/in.$^2$, did not show any ultraviolet fluorescent material (*4*). The results described here, as well as those of other workers, show that plastic and rubber laboratory apparatus usually considered chemically stable actually release undesirable substances under relatively mild conditions (*5*).

Though these fluorescent contaminants described here are not lethal to the growth of excised lemon fruit tissue in vitro, they may possibly exert adverse effects upon tissue growth. In addition, these contaminants make chromatographic analysis of nutrient solution for fluorescent material released from growing lemon fruit tissue more difficult. Consequently, all solutions of reagents, as well as glass-redistilled water, that I use for preparing nutrient solution are prepared in and handled with all-glass apparatus, and I grow excised lemon fruit tissue in all-glass vessels.

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2. Bakelite screw caps that have been subjected to repeated autoclaving still release this fluorescent material during autoclaving. When autoclaved on vials without any liquid contents, there is enough residual moisture resulting from steam condensation to contaminate the vials with this ultraviolet fluorescent material from the Bakelite caps. Glass-redistilled water also extracts ultraviolet-fluorescent material from Bakelite caps with or without liners within 24 hours at room temperature. Water conditioning or water treatment is a standard procedure used by steam plants to prevent corrosion of the steampipes. The steam plant at the University of California, Los Angeles, treats water used for steam production with a strongly alkaline morpholine solution (formula 485, Garratt-Callahan Co.) to inhibit steampipe corrosion. This morpholine solution fluoresces and contaminates materials autoclaved with steam from the steam plant. Therefore, I autoclaved all material in an autoclave that was equipped with a self-contained steam generator.
3. In a personal communication from Rhoades Rubber Corp., Easthampton, Mass., the following substances used in compounding black rubber stoppers were listed as possible fluorescent materials extracted with alcohols: (i) naphthenic oil; and (ii) 2-mercaptobenzothiazal tetramethyl thiuram monosulfide. Pure latex-type (pharmaceutical grade) rubber stoppers were kindly furnished by Rhoades Rubber Corporation.
4. The alcohols listed in this study were reagent grade chemicals factory-packaged in brown glass bottles sealed with Bakelite screw caps. All these solvents showed a slight fluorescence under ultraviolet light (3660 Å), possibly resulting from the contact of these solvents with the Bakelite caps. Consequently, all the alcohols used in this study were redistilled and stored in all-glass containers.
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2 August 1965

## Fatty-Tissue Changes in Rats with Acclimatization to Altitude

Abstract. *Adipose tissue in the adult white rat changes in both quantity and histologic characteristics during a 5-week period of acclimatization to a simulated altitude of 4350 meters at 26°C. These findings are descriptive and do not at present permit conclusions as to the mechanisms involved.*

Acclimatization to high altitude is a complex process that necessitates the adaptation of many tissues. There are no data regarding possible changes in adipose tissue during exposure in vivo to altitude, but species in which brown fat is found seem to have a greater capacity to survive under lower pressures of oxygen (*1*) than do species with less brown fat. A hypothesis that possession of brown fat contributes to tolerance of hypoxia might gain support if an increase in the quantity and distribution of brown fat could be demonstrated when animals are chronically exposed to reduced ambient oxygen in a thermoneutral environment.

Eight male, adult, Sprague-Dawley rats (286 to 304 g) were exposed, one per cage, to a simulated altitude of 4350 m for 5 weeks at 26°C (*2*); three of them did not survive. Another group of seven rats (284 to 307 g) was maintained as controls for 5 weeks at sea level at 36°C. All animals were then killed with Nembutal; brown fat from the superior cervical, interscapular, subscapular, pericardial, and axillary regions and white fat from the inguinal region were extirpated and

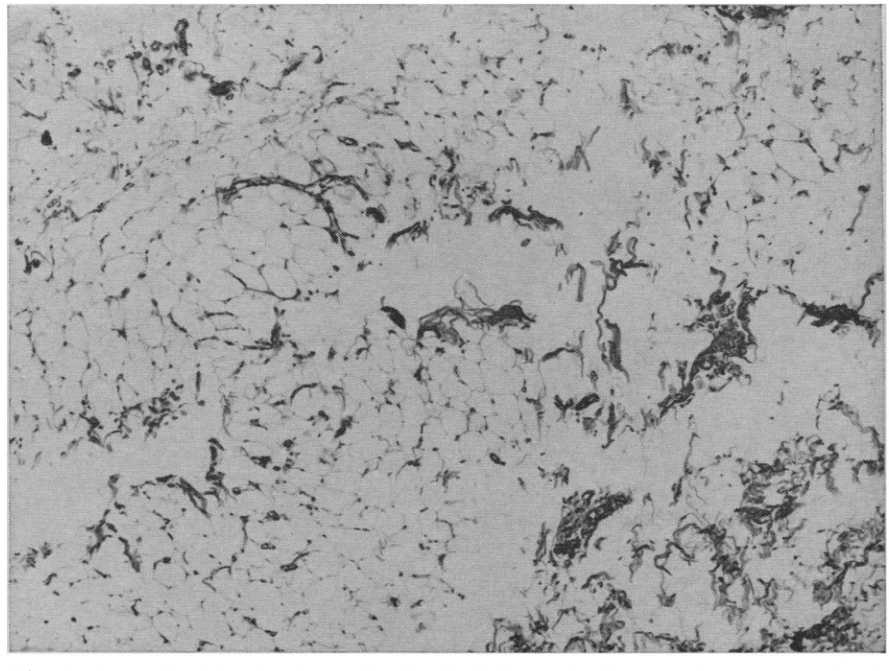

Fig. 1. Normal white fat from the inguinal fat pad of a rat kept at sea level; hematoxylin-eosin stain (× 100).