of the fiber and was not affected by the loss of contact between the myoplasm and the retraction clot that occurred in the fibers of small diameter. The part of the fiber adjacent to the injury lost excitability.

Some fibers were tetanized while the injured ends were being observed by microscope. Stimulation speeded up coagulation in the large fibers so that a series of retraction clots formed. In the smaller fibers the cones retained their shape during stimulation, and no new clots formed for a considerable time. This observation may be pertinent to the problem of the nature of the mechanical coupling between myoplasm and sarcolemma. The only organelle of the cell known to connect to the sarcolemma is a delicate transverse network of tubules at the level of the Z membrane in each sarcomere (T-tubule system) (*6*). If the myofibrils pull against such a network, one would expect to see a concave surface at the injured end. The cone suggests that a discarded model of muscle, consisting of a viscous gel enclosed in an elastic membrane, with tension transferred by short-range molecular forces, may have some validity (*4*).

Similar experiments incorporating injury were performed in 1951 (*6*); the decrease in active tension reported was probably due to loss of excitability.

In 1940 (*2*) we showed that the relation between length and passive tension in single muscle fibers was approximately the same for both the sarcolemma tube and the intact fiber. The injury experiments just described show that the tube can also transmit active tension; this, we think, occurs not only in the case of a complete myoplasmic break. Many diseases and injuries cause patchy pathological changes in muscle with the result that parts of a single fiber may be normal and parts abnormal. Such fibers from muscle that has been injured by section of one tendon (tenotomy) are often able to develop normal isometric tension. It is probable that tension developed in a normal part can be transmitted to the tendons by the sarcolemma, thus bypassing the damaged section.

In studies of normal muscle, this bypass must be considered in any analysis of the correlation of muscle length and active tension. It is an interesting possibility that the low metabolic cost of negative work (work done by a muscle while it is being stretched) may be due to the sarcolemma bypass rather than to a reversal of chemical reactions.

SIBYL F. STREET
ROBERT W. RAMSEY
Department of Physiology, Medical College of Virginia, Richmond

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27 May 1965

Iodination in Relation to Thyroglobulin Maturation and Subunit Aggregation

Abstract. *Noniodinated subunits of thyroglobulin can aggregate, but iodination of the aggregate is required for its stabilization (maturation). Rat-thyroid slices incorporate amino acids into subunits, but cannot form mature thyroglobulin from the newly synthesized subunits. This defect leads to an accumulation of 16S and 12S proteins, although the preexisting thyroglobulin is 19S. Accumulation of 16S and 12S proteins can be produced in rat thyroids by the administration to the animals of a thiocarbamide derivative, methimazole. Upon withdrawal of methimazole, iodination of the 16S and 12S proteins proceeds, and 19S protein appears.*

The iodination of thyroglobulin-like proteins in thyroid slices occurs after completion of the polypeptide backbone (*1–5*), but it is not known whether the presumed subunit precursors (3–8*S* and 12*S*) of thyroglobulin (19*S*) are normally iodinated before aggregation and whether iodination is essential for the aggregation. There probably exists an anatomical, as well as a temporal, separation of iodination and aggregation (*2, 6, 7*). In lamb-thyroid slices after incubation with a labeled amino acid or with ^{125}I, there are three classes of thyroglobulin molecules, each with slightly different physical properties. Iodine-labeled thyroglobulin (18.5*S*) is intermediate in rate of ultracentrifugal sedimentation and in ease of dissociation to its half-molecule (12*S*) when compared with amino acid–labeled thyroglobulin (17.5–18*S*) and with unlabeled, preformed thyroglobulin (19*S*) of the lamb-thyroid slice (*2, 6, 7*). These properties may reflect the relative biologic maturity of the three kinds of thyroglobulin. Iodination appears not to be necessary for subunit aggregation, since amino acid labeling of thyroglobulin occurs in lamb-thyroid slices despite complete inhibition of the incorporation of iodide into organic form by propylthiouracil (*1, 2*).

However, when thyroid slices are incubated for very brief periods with radioactive iodide, the 3–8*S* and 12*S* proteins are more highly labeled than the 19*S* material (*2, 4, 7*). The proportion of radioactivity associated with the subunit proteins decreases with continued incubation. It is not known whether these iodine-labeled smaller units represent breakdown products, derived from an especially labile fraction of thyroglobulin, or whether they are subunits which are iodinated as such and which may or may not undergo aggregation to form thyroglobulin.

In this report we present data which support the concept that iodination, while not essential for aggregation, is required for thyroglobulin maturation.

Rat-thyroid slices incorporate radioactive amino acid primarily into three proteins, all of which sediment more slowly than thyroglobulin (Fig. 1) (*8*). As has been shown for lamb- (*2, 7*) and for human- and calf-thyroid slices (*9*), radioactive amino acid is incorporated into 3–8*S* and 12*S* proteins before label appears in more rapidly sedimenting particles. However, unlike the situation in these other species in which label appears in 17.5–18*S* particles after about a 20-minute lag, in the rat radioactivity accumulates to a large extent in a 16–16.8*S* particle and continues

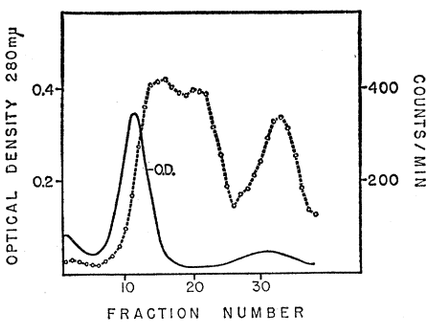

Fig. 1. Incorporation of ^{14}C-leucine into proteins of rat-thyroid slices. Thyroids from three rats were bisected and incubated in 2 ml of Eagle's medium containing 5 μc of ^{14}C-leucine ($4 \times 10^{-4}M$) at 37°C for 5 hours in 100 percent oxygen. The fraction of the soluble thyroid proteins precipitated by 0 to 50 percent ammonium sulfate was analyzed. The major optical-density peak is at 19*S*. The radioactive peaks are at 16*S*, 12*S*, and 4*S* from left to right along the gradient.

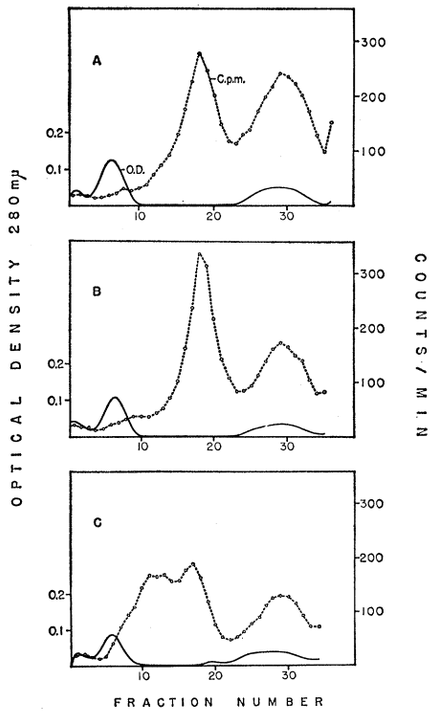

Fig. 2. Effect of continued incubation in the presence of unlabeled amino acid on the sedimentation of pulse-labeled rat-thyroid proteins. Thyroids from nine rats were bisected and incubated in 2 ml of Eagle's medium containing 80 μc of ^{3}H-leucine. After 10 minutes the slices were removed and rinsed in unlabeled media. One-third of the slices (*A*) were prepared for analysis as in Fig. 1; the remaining slices were further incubated in media containing unlabeled L-leucine ($10^{-2}M$) either for 30 minutes (*B*) or for 4 hours (*C*). The major optical-density peaks are at 19*S* and the highest radioactive peaks are at 12*S*. The fraction of the soluble thyroid proteins precipitable by 0 to 70 percent ammonium sulfate was used.

to increase in the 12*S* protein (Fig. 1). In striking contrast to the lamb-thyroid slice, considerable radioactivity persists in the 12*S* region but little accumulates in the 19*S* region, even when pulse-labeled rat-thyroid slices are further incubated with unlabeled amino acids (Fig. 2). The sequence of labeling of the 12*S* and 16*S* proteins and their relative content of radioactivity at different times make it unlikely that the labeled 12*S* particle is primarily a breakdown product of the 16*S* particle, or that the 16*S* particle is an artifactual aggregate of the 12*S* particle. The 16*S* protein is likely an asymmetric form of the 19*S* protein and similar to the 17*S* particle produced by heat or by alkaline treatment of native thyroglobulin (*10*). On the other hand, incubation of rat-thyroid slices with tracer amounts of ^{125}I and administration of ^{125}I to rats lead to the usual picture of labeling predominantly in the 19*S* region. Thus, the rat-thyroid slice, to an even greater extent than slices from the other species mentioned, is blocked in its ability to synthesize mature 19*S* thyroglobulin. This block in maturation is peculiar to the system in vitro in that the major soluble protein species already existing is 19*S*.

That the block may be due to a defect in iodination, or steps which follow it, is indicated by experiments in which iodination is completely inhibited by methimazole (*11*) administration. In these experiments, preexisting 19*S* thyroglobulin is lost from the gland presumably as a result of proteolysis induced by thyroid-stimulating hormone. When methimazole treatment is supplemented with thyroxine to block further proteolysis, proteins sedimenting at 16*S* and 12*S* accumulate (Fig. 3). Incubation of thyroid slices from such animals with a radioactive amino acid also leads to 16*S* and 12*S* labeling (Fig. 3*A*) as in the slices from untreated animals. These experiments indicate that the proteins labeled in thyroid slices from presumably normal rats are similar to those which accumulate in the thyroids of animals which are blocked in the organification of iodide and in the coupling of iodotyrosines. In the latter case noniodinated thyroglobulin precursor proteins accumulate in the gland in vivo. Further, the 16*S* protein, obtained from thyroids of treated animals or synthesized in vitro, can be readily dissociated to its 12*S* half-molecule. Labeled and unlabeled

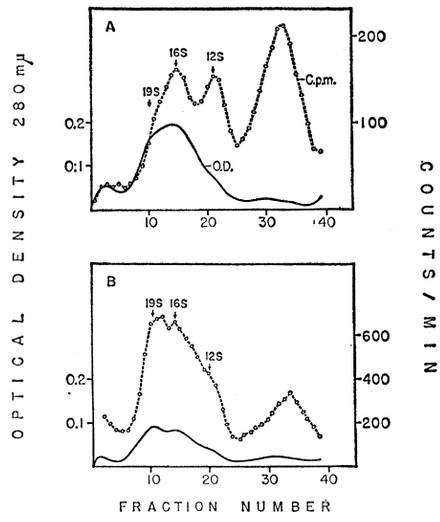

Fig. 3. Labeling patterns in thyroid slices from rats treated with methimazole and thyroxine. Eight rats were treated with methimazole for 20 days, and thyroxine was added for an additional 9 days. Thyroids from four rats were incubated in media containing 1 μc of ^{14}C-leucine for 5 hours (*A*), or in media containing 20 μc of ^{125}I for 30 minutes (*B*).

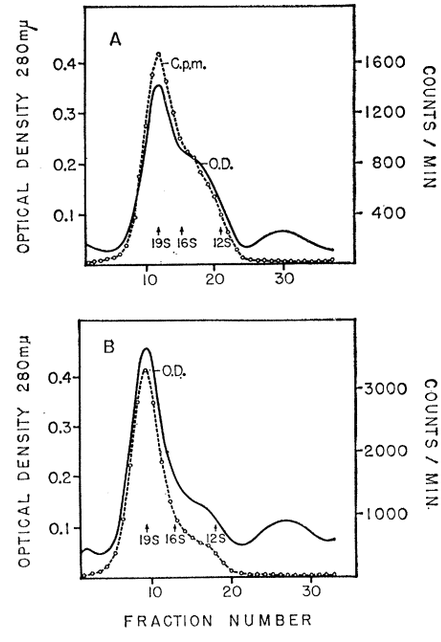

Fig. 4. Iodination in vivo of thyroid proteins of rats previously treated with methimazole and thyroxine. Four rats were treated successively with methimazole for 36 days, methimazole and thyroxine for 17 days, and finally thyroxine alone for 40 hours (*A*) or 96 hours (*B*). Each animal received 100 μc of ^{125}I intraperitoneally. The thyroids were removed after 30 minutes, and the soluble proteins were processed for centrifugation analysis.

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*).

RANDOLPH W. SEED
IRVING H. GOLDBERG
Department of Medicine, Harvard Medical School and *Beth Israel Hospital, Boston, Massachusetts.*

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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