Role of Triiodothyronine-I^{131} Purity in T-3 Tests. (26442)

NORMAN D. LEE AND VINCENT J. PILEGGI (Introduced by R. J. Henry)
Radioisotope and Iodine Sections, Bio Science Laboratories, Los Angeles, Calif.

This laboratory has been concerned with investigation of various thyrodiagnostic procedures based on *in vitro* partitioning of I^{131}-labeled triiodothyronine (T-3) between plasma or serum proteins and such formed elements as erythrocytes(1) or ion-exchange resin beads(2). A number of variables have been reported as affecting reproducibility(3, 4). In a series of studies designed to evaluate other factors responsible for day-to-day variation attention was focused on the T-3 preparation. This report is concerned with purity and stability of such preparations and their role in T-3 tests.

Methods. T-3 preparations were stored in the refrigerator. Working solutions were used in a standard partitioning test employing whole blood(1). Descending chromatography was carried out using Whatman #3 paper and 2-butanol:4% NH_4OH (3:1) as solvent. Prior to spotting the T-3 preparation was diluted with a carrier solution containing a mixture of non-radioactive iodoamino acids and iodide. Strips were dried after development and surveyed with a radiochromatogram scanner (Scanogram II, Atomic Accessories) and the proportions of radioactive components were determined by planimetry. Identification of components was based on coincidence of radioactivity with spots shown by spraying the strips with a ceric-arsenious acid reagent and counterstaining with methylene blue(5).

Results. Table I shows uptake values for 4 different whole blood specimens in the standard partitioning test when T-3 from 3 different commercial suppliers was used. When compared to normal values(1), these were interpreted as consistent with hypo-, eu-, or hyperthyroidism, depending on which stock T-3 preparation had been used. It could only be concluded that these differences arose from heterogeneity of the radioactive materials. This was demonstrated chromatographically; scanning records of radiochromatograms are shown in Fig. 1.

TABLE I. Role of Different T-3 Preparations.

T-3 from source	% uptake/100 hematocrit			
	Blood 1	Blood 2	Blood 3	Blood 4
A	17.2	17.0	14.1	13.3
B	13.8	14.3	8.8	10.4
C	6.7	6.9	4.5	5.5

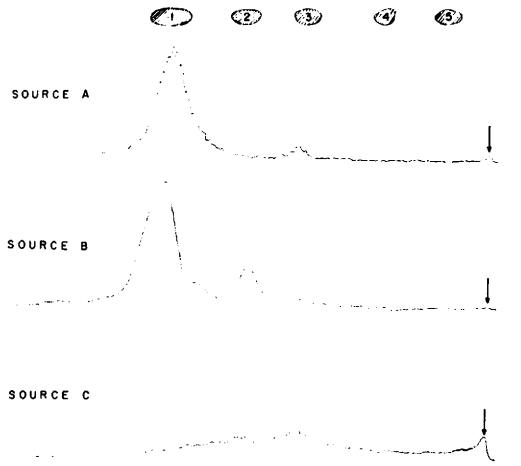

FIG. 1. Radioactive scanning records of chromatographic analysis of T-3 preparation from 3 different sources. Numbered spots at top are from a ceric-arsenious acid stained chromatogram of a mixture of iodoamino acids. (1, triiodothyronine; 2, thyroxine; 3, iodide; 4, monoiodotyrosine, and 5, diodotyrosine. Arrows mark origin of each strip.)

These findings prompted chromatographic analysis of a number of samples obtained from Source A; results are presented in Table II. These samples were all reasonably new. Excepting preparation #4 they can be seen to be highly uniform, being only minimally contaminated with radioactive materials other than T-3. Non-iodide impurities were also rather uniform, being distinguishable only as a deformity in one shoulder of the T-3 peak. Preparation #4 had been delayed in transit, hence its increased proportions of impurities may have arisen during this period.

Another factor is appearance of radioactive impurities with age. Data presented in Table III are concerned with 3 preparations

TABLE II. Composition of Various Fresh T-3 Preparations.

T-3 prep. No.	Age, days	I^{131} distribution, % T-3	I^-	Other constituents
1	0	98	2	None
2	1	100	0	Less than 1%
3	3	100	0	*Idem*
4	4	88	4	8%
5	4	98	2	Less than 1%

which had minimal impurities on receipt. Although kept under identical conditions quantitative aspects of development of impurities seemed to be distinctly different. Time intervals employed were within or only slightly beyond the accepted usable life of such radioactive preparations.

Discussion. It was observed that there were marked differences in radiopurity of T-3 preparations obtained from different sources, that markedly impure preparations were obtained occasionally from sources having a record of high purity, and that some pure preparations developed significant proportions of impurities over a short time interval. Only the first of these problems may be directly traceable to source of supply; time and temperature of shipment, particularly where unusual delays are involved, may be responsible for the second and third observations. Nevertheless, these phenomena certainly explain the findings shown in Table I and may account for spurious results occasionally experienced with this type of test. Chromatographic monitoring of each lot of T-3 on receipt may be a sufficiently simple procedure for routine practice. We have found use of a standard lyophilized or pooled serum of additional value as a monitoring device for each run. This is possible with any modification of the T-3 test using only patient's serum or plasma and an adsorbent other than patient's own erythrocytes.

TABLE III. Effect of Preparation Age on Proportions of Various I^{131} Constituents.

T-3 prep. No.	Age, days	I^{131} distribution, % T-3	I^-	Other constituents
1	0	98	2	None
	6	96	4	Less than 1%
	18	91	2	7%
3	3	100	0	Less than 1%
	20	85	3	12%
2	1	100	0	Less than 1%
	6	90	1	9%

Summary. Using a standard thyrodiagnostic test based on *in vitro* partitioning of triiodothyronine-I^{131} between plasma proteins and erythrocytes of whole blood, results were found to differ depending on the commercial source of labeled triiodothyronine. Chromatographic study showed these preparations to be heterogeneous. Impurities were occasionally present in fresh materials and developed in significant proportions during the accepted usable life of the preparation.

1. Hamolsky, M. W., Golodetz, A., Freedberg, A. S., *J. Clin. Endocrin. and Metab.*, 1959, v19, 103.

2. Sterling, K., Tabachnick, M., *ibid.*, 1961, April, in press.

3. Meade, R. C., *ibid.*, 1960, v20, 480.

4. Adams, R., Specht, N., Woodward, I., *ibid.*, 1960, v20, 1366.

5. Mandl, R. H., Block, R. J., *Arch. Biochem. Biophys.*, 1959, v81, 25.

Received February 8, 1961. P.S.E.B.M., 1961, v106.

Fluorescent Antibody Stainability and Other Consequences of the Disruption of Mycobacteria. (26443)

CHARLES C. SHEPARD AND DAVID KIRSH

Communicable Disease Center, Public Health Service, U. S. Department of HEW, Atlanta, Ga.

During the last few years a number of attempts in this laboratory to stain mycobacteria with fluorescent antibody have been unsuccessful, and the lack of publication of successful efforts by others suggests a similar experience elsewhere.

The mycobacteria are characterized by a high lipid content and their lipophilic be-