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THE EFFECT OF STORAGE TIME AND TEMPERATURE ON THE T-3 UPTAKE AND T-4 TESTS FOR THYROIDAL HORMONES

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ABSTRACT

Serum and clotted blood from nine subjects were subjected to different conditions of temperature and storage times for up to two weeks to imitate the shipment of blood samples to a reference laboratory for T-3 uptake and T-4 tests. The T-3 uptake and T-4 values were not affected by the time or temperature conditions, except in the case of the whole blood, which developed gross hemolysis. Gross hemolysis gave lowered T-4 values, while not affecting the T-3 uptake values.

INTRODUCTION

This investigation examines the effect of storage time and temperature on samples collected for T-3 uptake and T-4 test, such samples being treated to various conditions of temperature and storage time. Blood samples are often shipped in the original collection tube in a clotted state by bus or mailed from rural physicians to a reference laboratory often thousands of miles away. The conditions to which the samples are subjected are variable and often involve a considerable lapse of time until they are shipped, or until the actual testing is completed.

The T-3 uptake test measures the unsaturated binding capacity of thyroid binding globulin (TBG). This test uses the physical-chemical principle of surface absorption, which is essentially time and temperature independent according to Nusynowitz (1970). The T-4 test measures the thyroxine bound to the TBG based on the radioisotopic principle of competitive protein binding.

MATERIALS AND METHODS

Blood samples from five males and four females were collected for T-3 uptake and T-4 test using the TRI-TAB and TETRA-TAB test kits (Nuclear Medical Laboratories, Inc., Dallas, Texas). Blood samples were allowed to clot at room temperature for one hour. Serum was separated from the formed elements by centrifugation for 15 minutes at 700 x g.

Aliquot samples of serum were stored as follows: frozen (−20°C), refrigerated (4°C), room temperature (25°C), and elevated temperature (40°). Clotted samples were also treated to the same storage conditions in the original collection tube. Clotted samples were not frozen, due to the obvious gross hemolysis which would develop. After the initial assay, each sample aliquot was tested for T-3 uptake and T-4 analysis on the day of collection and at 24 hours, 48 hours, 72 hours, 96 hours, one week and two weeks after collection.

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RESULTS

Visual observation of all serum samples which were incubated for T-3 uptake and T-4 analysis indicated no visible change in the serum appearance. Clotted blood incubated at 40°C produced increasing hemolysis after 72 hours. Following two weeks' incubation, the hemolysis was marked. Serum from clotted blood incubated at 25° and 4°C was not altered in appearance from the initial sample.

T-3 values in serum removed from the clot within one hour after collection and storage at 40°, 25°, 4°, and −20°C for up to two weeks were not significantly changed and were within normal limits (Table I). T-3 values in serum removed from the clot following incubation of the whole blood for up to two weeks at 40°, 25°, and 4° were not significantly changed and were within normal limits (Table II).

T-4 values in serum removed from the clot within one hour after collection and stored at 40°, 25°, 4°, and −20° for up to two weeks were not significantly changed and were within normal limits (Table III). T-4 levels in serum removed from the clot following incubation of the whole blood for up to two weeks at 25° and 4°C were not significantly changed (Table IV). However, there was a highly significant difference between the means of untreated serum and the means of serum stored on the clot for 96 hours, one week and two weeks when incubated at 40°C.

DISCUSSION

The manufacturer of the TRI-TAB and TETRA-TAB tests cautions that while a small amount of hemolysis does not affect the T-4, sera containing greater than 100 mg hemoglobin/100 ml tend to yield a decreased value. Our data supports these findings. After 48 hours' incubation of clotted blood, the sera contained 36.80±8.05 mg hemoglobin/100 ml. The T-4 levels were reduced in these sera, but not significantly. Following 72 hours' incubation of clotted blood, the sera contained

180±34.86 mg hemoglobin/100 ml and the T-4 levels were significantly ($p<0.01$) reduced.

In order to determine the cause of the reduction in the T-4 levels by hemolyzed sera, blood clots were hemolyzed after removal of the sera to obtain the gross hemolysis observed. There was no detectable T-4 in the hemolyzed erythrocytes when assayed by the TETRA-TAB procedure. The hemolysate was then mixed with sera containing known T-4 values and assayed. The T-4 values were significantly ($p<0.01$) reduced. In a separate experiment, ^{125}I labeled T-4 was added to the hemolyzed sample and then assayed for T-4. The supernatant from the extraction step which contained the protein retained 35% (±1.6) of the ^{125}I labeled T-4. The supernatant produced when the silicate particles were separated following the incubation phase retained 47% (±2.5) of the ^{125}I labeled T-4. Based on these data, it appears that an intracellular constituent of erythrocytes, probably the hemoglobin molecule, binds T-4, thus reducing the T-4 to be absorbed by the silicate particles.

Sera for T-3 uptake were not affected by temperature or storage time up to two weeks. This extends the stability time for T-3 using the TRI-TAB procedure by seven days. Desaty and Green (1975) reported stability up to seven days in sera tested for T-3 uptake and T-4 at −20°, 4°, and 23°C. Increasing hemolysis, as seen in whole blood incubated at 40°C for two weeks, did not significantly alter the T-3 uptake.

Based on these data, it appears that the serum used for the assay of T-4 should be free of hemolysis, while T-3 uptake is not affected by the hemolysis. Due to erroneously low T-4 values obtained from hemolyzed sera, these sera should not be accepted for assay.

TABLE 1: *The Effect of Storage Time and Temperature on Triiodothyronine (T-3) Uptake In Serum Removed from the Clot. Values Expressed as Percent Uptake of I^{125} Labeled T-3.*

Storage Time	Storage Temperature			
	40°C	25°C	4°C	−20°C
Initial		38.24±0.78		
24 Hours	35.97±0.68	37.53±0.69	37.96±0.56	37.93±0.82
48 Hours	37.14±0.79	38.56±0.88	39.77±0.84	38.84±0.82
72 Hours	36.63±0.57	37.42±0.88	38.93±0.79	37.71±1.09
96 Hours	37.86±0.75	37.83±1.13	39.61±0.98	38.51±0.98
1 Week	38.84±1.13	39.35±0.96	39.88±0.80	38.35±1.27
2 Weeks	40.15±1.11	39.15±0.95	38.47±0.85	38.58±0.74

Values represent the mean ± SE of nine individual patients, assayed in duplicate.
Significant difference between means at the 0.05 (*) or 0.01 (**) level of confidence as determined by *t* test.

TABLE II: *The Effect of Storage Time and Temperature on Triiodothyronine (T-3) Uptake in Serum Stored on the Clot. Values Expressed as Percent Uptake of I^{125} Labeled T-3.*

Storage Time	Storage Temperature		
	40°C	25°C	4°C
Initial		38.24±0.78	
24 Hours	35.21±0.71	37.57±0.79	38.46±0.62
48 Hours	35.94±0.69	38.12±0.70	40.10±0.98
72 Hours	34.98±0.81	37.30±0.87	41.35±0.61
96 Hours	35.45±0.94	38.65±0.65	38.88±1.04
1 Week	35.70±1.02	38.55±1.42	38.91±1.04
2 Weeks	35.60±1.23	38.67±0.80	38.72±0.56

Values represent the mean ± SE of nine individual patients, assayed in duplicate.
Significant difference between means at the 0.05 (*) or 0.01 (**) level of confidence as determined by *t* test.

TABLE III: *The Effect of Storage Time and Temperature on Thyroxine (T-4) Levels in Serum Removed from the Clot. Values Expressed as μg T-4 per 100 ml.*

Storage Time	Storage Temperature			
	40°C	25°C	4°C	−20°C
Initial		7.41±0.53		
24 Hours	7.27±0.27	6.82±0.29	7.05±0.26	6.91±0.21
48 Hours	7.11±0.32	6.95±0.34	6.50±0.34	6.30±0.69
72 Hours	7.54±0.30	7.48±0.35	6.84±0.30	6.98±0.31
96 Hours	7.10±0.26	7.05±0.24	6.55±0.22	6.57±0.25
1 Week	7.40±0.25	7.23±0.36	6.85±0.37	6.55±0.30
2 Weeks	7.65±0.31	7.45±0.27	6.62±0.40	6.41±0.27

Values represent the mean ± SE of nine individual patients, assayed in duplicate.
Significant difference between means at the 0.05 (*) or 0.01 (**) level of confidence as determined by *t* test.

TABLE IV: *The Effect of Storage Time and Temperature on Thyroxine (T-4) Levels in Serum Stored on the Clot. Values expressed as μg T-4 per 100 ml.*

Storage Time	Storage Temperature		
	40°C	25°C	4°C
Initial		7.41±0.53	
24 Hours	7.07±0.35	7.06±0.28	6.83±0.36
48 Hours	6.26±0.34	6.82±0.33	6.81±0.39
72 Hours	6.02±0.45*	7.20±0.32	6.71±0.35
96 Hours	5.40±0.23**	6.95±0.24	6.67±0.31
1 Week	4.04±0.27**	6.96±0.30	6.51±0.32
2 Weeks	4.14±0.50**	6.58±0.26	6.26±0.23

Values represent the mean ± SE of nine individual patients, assayed in duplicate.
Significant difference between means at the 0.05 (*) or 0.01 (**) level of confidence as determined by *t* test.

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