C^{14} Thyroxine Transport in Thoracic Lymph in Rats.* (23410)

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(Introduced by James J. Smith)
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The thyroid hormone must become part of the cell's environment to exert its peripheral metabolic action. The entrance of the hormone into the circulation and subsequent binding must therefore be followed by its escape into the extravascular extracellular fluid which bathes peripheral cells. Salter *et al.*(1) and Salter and Johnson(2) emphasized the importance of binding "hormonal iodine" to the smaller molecular sized protein fractions of plasma and these would probably escape through the capillaries into the lymph carrying with them relatively rich amounts of "hormonal iodine" for the peripheral cell environment.

The present study is concerned with the transport mechanism and character of the hormone in thoracic lymph using DL-thyroxine-l-C^{14}.†

Methods. Adult male rats of the Sprague-Dawley strain weighing about 225 g were used. All animals were maintained on Rockland Complete Rat Diet and fed *ad libitum*. Under ether anesthesia, the thoracic lymph duct was cannulated through a left lateral abdominal incision using a polyethylene tube (inside diameter, 1.10 mm) according to a modification(3) of Bollman's technic(4). The abdominal opening was closed and sealed with collodion and the animal placed in a restraining cage. When the lymph reached the end of the tube (volume of 0.2 ml), the animal was injected subcutaneously in the right flank with 780 μg (one μM) of DL-thyroxine-l-C^{14}/kg body weight. From 8 animals, 8 hourly lymph samples were collected in 2 ml pipettes which had been previously cut off at the lowest graduation and closed with a rubber-dam. One drop of heparin was added to each collecting pipette to prevent coagulation of the sample. Lymph radioactivity was determined by drying 0.1 ml aliquots on copper plates and counting them under a thin mica window Geiger counter. *Plasma radioactivity levels* were determined in 8 animals from blood collected by 8 hourly heart punctures. About 0.4 ml of blood was obtained at each collection period and duplicate 0.05 ml samples of plasma were plated and counted in the same manner as for lymph. *Chromatographic analysis* of lymph was made using modification of the method described by Rosenberg(5). Cellulose columns 85 cm in height were prepared using Solka-Floc BW 200. The running solvent contained n-butyl alcohol, glacial acetic acid, and water in proportions of 75:10:15, respectively. Regulation of the solvent flow rate to 0.5 ml/hour was attained by connecting the 300 ml solvent container to top of column. Pooled butyl alcohol extracts of the most active samples (2-4 hours) of lymph were used. Approximately 1 to 2 μM each of thyroxine, triiodothyronine and diiodotyrosine was dissolved in 0.1 ml of running solvent and added to top of column as a carrier and for position identification. Effluent samples of 0.5 ml were obtained using an automatic fraction collector and radioactivity measurements were made in the same manner as for lymph and plasma. The presence and relative concentrations of each of the known constituents were determined by the photometric ninhydrin method of Moore and Stein(6).

Results. From Fig. 1 it is seen that over 3% of the injected dose of C^{14} labelled thyroxine appeared in the thoracic lymph after 8 hours. There is nearly a straight line relationship between the accumulation of activity and time.

Fig. 2 compares the radioactivity present in plasma and lymph. Radioactive thyroxine appeared in both fluids within the first hour, peaking in about 1-3 hours, and then grad-

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† The C^{14} used was obtained from Oak Ridge National Laboratory on allocation from Isotopes Division, U. S. Atomic Energy.

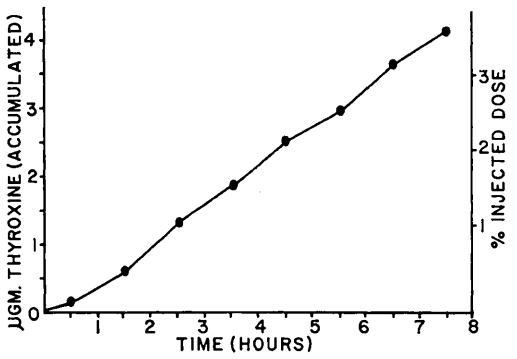

FIG. 1. Accumulated lymph radioactivity after a single subcut. inj. of 0.78 mg/kg of thyroxine C^{14}.

ually declining over remainder of the collection period. There is a slight delay in appearance and peak of activity in the lymph when compared with plasma. The amount of C^{14} thyroxine in the plasma remained higher than in the lymph throughout the 8 hour experimental period. The plasma level showed an increase of 2 to 4 times that of lymph, during the first 2 hours, after which time this difference was gradually reduced.

Fig. 3 shows concentration of thyroxine/g of plasma and lymph protein. These values were calculated on the basis of determinations of total protein content of these respective fluids at zero time (6.13 g % for plasma; 3.51 g % for lymph). This method requires some discussion. To follow the plasma and lymph concentrations of thyroxine for an 8 hour period, a fairly large amount of protein fluid was removed from the rat. Approximately 3.12 ml of whole blood was withdrawn, and the entire lymph flow in this time interval, about 5-6 ml was collected. These withdrawals were of course performed on 2 different groups of animals, because a single animal could not have sustained both hemorrhage and lymph deprivation. Because of this, it would seem that to calculate thyroxine concentration relative to initial protein levels would be invalid. According to Drinker and Field(7) however, there is a close correlation between plasma and lymph protein content. Thus, drainage of lymph will not only reduce its own protein concentration, but will effect a proportional lowering of plasma protein concentration; hemorrhage, likewise, will cause a hemodilution and thus a reduction in plasma proteins, and at the same time will induce a parallel decrease in lymph proteins. Calculation of thyroxine concentration/g of zero hour protein concentration, should reflect the relative changes that take place over a period of time, since both fluids will vary their protein concentration in a similar manner. In Fig. 3 then, the actual concentrations of

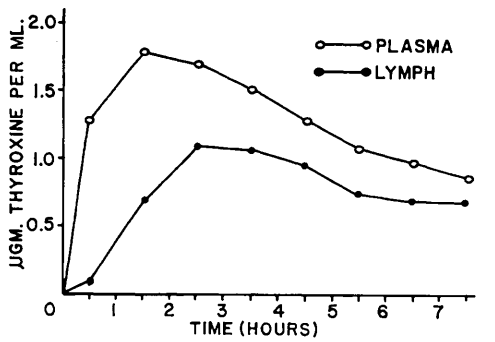

FIG. 2. Concentration of thyroxine C^{14} in plasma and lymph after a single subcut. inj. of 0.78 mg/kg.

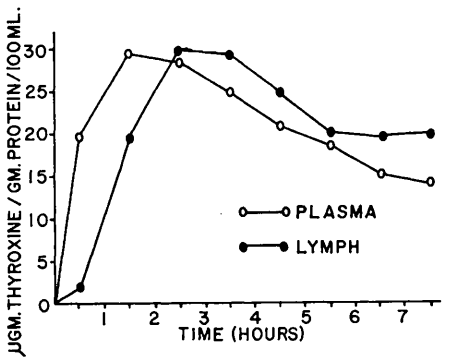

FIG. 3. Concentration of thyroxine C^{14}/g of plasma and lymph protein after a single subcut. inj. of 0.78 mg/kg.

thyroxine/g of protein are undoubtedly higher in the later hours than that shown, since the values are determined on the basis of initial protein content. But the relative changes in concentration remain the same. Fig. 3 indicates that after the 4th hour the lymph contains 30% more thyroxine/g of protein than does the plasma. Salter believes this is due to selective leaking of the small albumin and alpha-1 globulin molecules, the carriers of thyroxine, from the capillaries into the interstitial fluid. Salter found lymph to contain twice the amount of thyroxine as plasma when

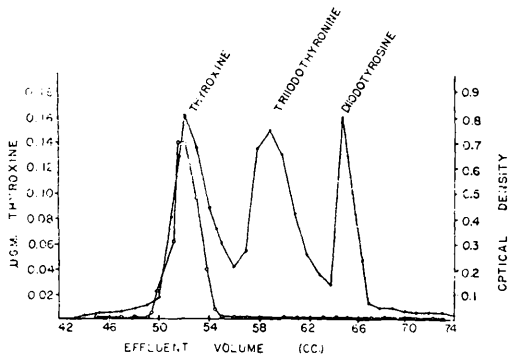

FIG. 4. Cellulose chromatographic analysis of butanol extracts of pooled (2 to 4 hr) lymph samples. (Solid dots represent known materials; open circles, material present in lymph extracts.)

calculated on a per gram protein basis; this may be due to the fact that he obtained his lymph from the cervical lymphatics, a region in which the A/G ratio is considerably higher than in thoracic duct lymph. Thoracic duct lymph has a varied origin, obtaining its fluid from the lower extremities and from abdominal organs. The liver is responsible for much of the duct's lymph, and since, according to Starling(8) the porous capillaries of the liver, unlike those of other tissues, allow all but the largest globulin molecules to pass through, the lowered A/G ratio of the thoracic duct lymph is no doubt due to the flow of beta globulin from the liver. Since thyroxine is largely associated with the albumin and alpha-1 fraction of lymph protein, the increase in beta globulin would reduce the concentration of thyroxine when expressed/g of protein. Thus, a 30% elevation of thyroxine/g of protein in duct lymph over plasma lymph would be significant, and would tend to substantiate Salter's findings.

The results of cellulose chromatographic analysis of butanol extracts of pooled lymph samples collected from 2 to 4 hours after injection are shown in Fig. 4. The radioactive peak of the lymph extracts appeared to be well within the limits of the known thyroxine and therefore identified as such. These results indicate that the butyl extractable radioactivity present in the lymph after C^{14} thyroxine administration represents unaltered thyroxine. It therefore seems likely that thyroxine, released into the circulation from the thyroid gland during this early period (2-4 hr), is bound to the smaller molecular sized protein fractions of the plasma which preferentially escape into the extravascular fluid transporting to the peripheral cell environment "hormonal iodine" in the form of unaltered thyroxine. Although no analysis of the 8 hr lymph sample was made it is possible that by this time a significant amount of administered C^{14} thyroxine could have been deiodinated by the peripheral cells and C^{14} triiodothyronine would appear in the lymph. The level of triiodothyronine in any case would remain low as it is less firmly bound to the plasma protein and readily displaced by thyroxine(9) and consequently rapidly eliminated in the urine, allowing a minimum of recirculation.

Summary. Plasma and thoracic lymph concentrations of thyroxine-l-C^{14} were followed in normal male rats after a single subcutaneous injection of 780 μg/kg body weight. Approximately 3% of administered dose appeared in the lymph during the 8 hour experimental period. The level of radioactivity in the lymph was lower than the plasma on a volume basis but significantly higher if calculated on the basis of protein content. Cellulose column chromatographic analysis of lymph samples taken between 2 and 4 hours, revealed the radioactivity present to be unaltered thyroxine.

1. Salter, W. T., White, W. F., and McKay, E. A., *Fed. Proc.*, 1946, v5, 90.

2. Salter, W. T., and Johnston, M. W., *J. Clin. Endocrinol.*, 1948, v8, 911.

3. Reid, W., and Beckman, H., *Marquette M. Rev.*, 1955, v20, 116.

4. Bollman, J. L., Cain, J. C., and Grindley, J. H., *J. Lab. and Clin. Med.*, 1948, v33, 1349.

5. Rosenberg, I. N., *J. Clin. Endocrinol.*, 1951, v11, 1063.

6. Moore, B., and Stein, W. H., *J. Biol. Chem.*, 1948, v176, 367.

7. Drinker, C. K., and Field, M. E., *Lymphatics, Lymph and Tissue Fluids,* Williams and Wilkins Co., Baltimore, 1933.

8. Starling, E. H., *On the Fluids of the Body,* W. T. Kenner Co., Chicago, 1909.

9. Deiss, W. P., Albright, E. C., and Larson, F. C., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 513.

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