two and three hours after the administration were studied by thin layer chromatography. Fractions of free T_4, T_3 and iodine were colored by the Gmelin's F. F. C. A. method and counted in a well counter. Rf values observed were 0.55 for T_4, 0.45 for T_3 and 0.35 for iodine respectively. In groupI, T_4 showed the highest counts per minuite one hour after the administration. T_3 was found to be the lowest in all specimens.

Iodine increased significantly at two and three hours. In group II, radioactivity of iodine was always constant by the hour and significantly higher than those in group I or II. T_3 was higher than iodine at the second hour only. Scarce radioactivity was detected in T_4 of all samples from group II. In group III, Iodine showed the highest counts at the first hour and decreased rapidly thereafter. Comparing to Iodine, T_4 and T_3 were rather scarce. It was confirmed (1) that when rats were administered with ^{131}I-T_4, ^{131}I-T_3 or ^{131}I-Na intravenously, free T_4, T_3 and I were excreted the most into the bile during the first three hours, (2) that the excretion into the bile and deiodination of T_4 were carried out the most rapidly, and also T_3 could be distributed into tissues very quickly, and (3) that T_4 was excreted into the bile and deiodinated more slowly than T_3.

Studies on the Method of Determination of Free Thyroxine Concentration in Serum

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In the recent years it has come to well known that the peripheral action of the circulating thyroxine is due to a function of the free thyroxine.

Most of previous methods of the determination of free thyroxine are too complicated.

In the present study dialysis method was used for the determination of free thyroxine concentration, and the dialysate was analyzed for ^{131}I-iodide and ^{131}I-thyroxine by gel filtration on Sephadex G-25 column.

Dialysis:

The dialysis bag, Visking cellulose tube, containing 3 ml of serum and ^{131}I-thyroxine (0.015~0.03 μg) was dipped into an equal amount of isotonic phosphate buffer (pH 7.4) solution in the dialysis chamber.

The dialysis was performed in the dialysis chamber with mild shaking at 37°C and for 18 hours.

After dialysis the surrounding dialysate was analyzed for ^{131}I-iodide and ^{131}I-thyroxine by gel filtration.

Gel filtration:

Sephadex G-25 column is 8 cm in height and 0.6 cm in diameter.

The dialysate was flowed into this column and 10 ml of water was added to the column. The ^{131}I-iodine was eluted from the column with this water.

Then, for elution of the ^{131}I-thyroxine, 3 ml of normal serum was applied to the column.

The ^{131}I-thyroxine in dialysate, namely ^{131}I-free thyroxine, was expressed as a percentage of the total in the serum, and the free thyroxine iodine was calculated from the percentage of the free thyroxine and PBI.

No overlapping in the free thyroxine iodine volume was proved between normal and thyroid disorders.

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