that such data can be profitably employed in cases of doubtful paternity in situations where a tasting child is born to a non-tasting mother.

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The Use of I^{131} Red Cell Plasma Ratio as a Measure of Thyroid Function.* (18570)

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Chemical analyses have shown that the normal chloride content of the red blood cells is approximately 190 mg % and that of plasma approximately 365 mg %(1). This gives a ratio of 0.51 for $\frac{\text{RBC chloride}}{\text{plasma chloride}}$. Similar analyses of cells and plasma in persons treated with bromides give a similar ratio for RBC bromide/plasma bromide(2). It has been noted that chloride, bromide and iodide pass rapidly across the red cell membrane *in vitro* and there is no reason to doubt that equilibrium is reached rapidly *in vivo*, as well(3). Rall *et al.* have shown from *in vitro* studies in human blood that the red cell plasma ratios for iodide and chloride were 0.67 and 0.59, respectively(3). As would have been expected from a consideration of the chemical similarities and mean ionic radii of iodide as well as of chloride and bromide ions, we have noted from *in vitro* studies that iodide ion does give an RBC/plasma ratio similar to that of the other two halides, namely about 0.5(4,5). It has been well established that the degree of conversion of inorganic iodine to protein-bound iodine and the appearance of the latter in the blood stream is an index of thyroid activity. I^{131} has been used as a tracer by a number of investigators to follow protein-bound iodine levels of the plasma and its relationship to types of thyroid disease(6,7). In the past, it usually has been necessary to employ laborious analytical methods to obtain a reliable separation of the protein-bound iodine in the plasma from the inorganic iodine fraction.

In the studies to be described in this report, it was found that inorganic iodine penetrated the red cell rapidly. However, the cell membrane apparently is relatively impermeable to large protein molecules containing labeled iodine. Therefore, the concentration of iodine in red cells, using I^{131} as a tracer, may be used as an index of the inorganic iodine content in the blood. The red cell activity will, therefore, indicate how much of the total plasma activity is due to inorganic iodine and how much is protein bound. The varying red cell plasma ratio at different times following a test dose of I^{131} is the result of the organic binding of I^{131} by the thyroid and/or the removal of protein-bound iodine from

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the blood stream by the body.

Methods. Clinical evaluation: Carrier-free I^{131}, as supplied by the Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tenn., was given orally in 50 cc of water as sodium iodide with a small amount of $NaHSO_3$ (.015 N base). Test doses given ranged from 100 to 150 microcuries. The patients described in these studies were diagnosed upon clinical grounds. The diagnosis was confirmed with the usual laboratory tests indicated in the diagnosis of thyroid disease. This included an estimation of the basal metabolic rate, blood cholesterol level in patients suspected of hypothyroidism, and thyroid uptake studies using I^{131}, as well as the blood studies cited here. Larger amounts up to 10 millicuries were administered for therapy. It should be noted that the exact quantity given did not alter the ratios observed. The separation of red cells and plasma was made by centrifuging 5 cc samples of heparinized blood at 3000 r.p.m. for 30 minutes and transferring 2 g portions of red cells and plasma into separate dishes. A few drops of 95% ethyl alcohol were added to each sample to aid in obtaining a flat film which is necessary for consistent geometry. The samples were dried at 75°C for 8 to 24 hours to remove moisture. It was found that I^{131} was not lost by this procedure. After assay of the activity with a G.M. counter, corrections were made for self-absorption. The data are expressed as counts per second. The counting efficiency was approximately 10% of the total number of disintegrations. The ratio of the I^{131} in the red cell/I^{131} in the plasma is not influenced by losses of iodine from the blood either by thyroid uptake or urinary excretion since *in vivo* studies have shown that the equilibrium between the plasma and the red cells is rapidly established. Therefore, a change in the red cell-plasma ratio is a reflection of the presence in the blood of iodine-bound into large molecules which are not able to penetrate the red cell membrane. Presumably the prosthetic groups of these large molecules are iodine-labeled thyroxine and diiodotyrosine. The rate at which these iodine containing molecules are built up in the plasma as well as the total amount present has been related to the synthetic ability of the thyroid(8). For this reason the rate which the red cell, plasma ratio deviates from the value seen with inorganic iodine as well as the total deviation is an expression of thyroid function and tissue utilization of I^{131} tagged materials.

Results. The initial red cell ratios taken 1 to 3 hours after oral I^{131} administration represent inorganic iodine red cell plasma ratios in all groups which presumably was too brief a time interval for measurable amounts of protein-bound iodine to appear in the blood stream. The ratios of euthyroid and hyperthyroid were similar and varied from 0.42 to 0.52 and showed no initial relationship to the thyroid state of the patient. However, with the passage of time the ratios were observed to drop to values as low as 0.05 this particular value being observed at 80 hours after I^{131} administration to a patient suffering from extreme hyperthyroidism. A ratio of 0.05 suggests that only twice this concentration of inorganic iodine is in the plasma and therefore the plasma activity is 90% due to protein-bound iodine. Using the methods described above, blood samples from 13 patients have been studied with the following results.

Two of the patients judged to be hypothyroid showed little or no reduction of their inorganic ratio during the time interval studied which was 80 and 140 hours. The data obtained upon the hypothyroid patients gave half-times of greater than 100 hours when expressed as percent of the original ratio. The meaning of half-time for these purposes is merely an arbitrary expression of the time required for the ratio to drop from its initial value of 0.5 to 0.25. A ratio of 0.25 suggests that one-half of the iodine in the blood stream is bound into molecules which are too large to penetrate the red cell membrane as does inorganic iodine.

Similar studies upon 4 euthyroid patients demonstrated the ability of their thyroids to return greater amounts of the I^{131} test dose

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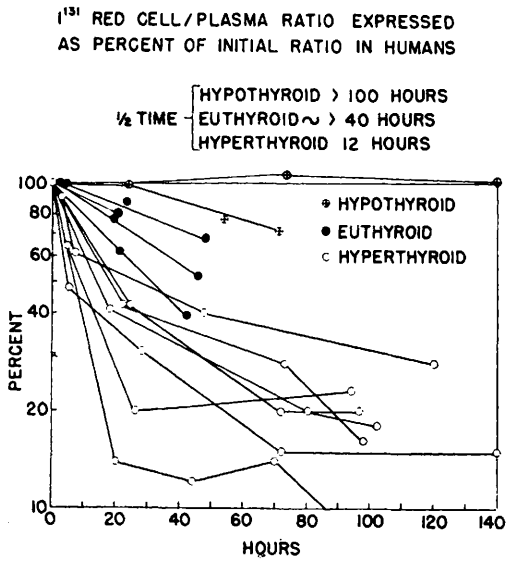

FIG. 1.

to the blood stream in organic combination, with a half-time of about 40 hours. Individual variations approached both hypo and hyperthyroid states. Seven hyperthyroid patients had red cell-plasma ratios which were lower than normal and appeared to be related to the degree of hyperthyroidism. Similar ratios were obtained upon hyperthyroid patients whether they were given test doses of I^{131} or therapeutic doses for the treatment of their disease during the periods reported here. These data are summarized in Fig. 1. It is noted that the ratios of hyperthyroids and euthyroids show greatest variation from each other 24 hours after I^{131} administration.

For this reason, a blood sample taken at this time, after a test dose of I^{131}, should give adequate information with respect to the rate of formation of protein-bound iodine and its deviation from normal. Blood samples from persons suspected of hypothyroidism gave more fruitful information when taken 35 to 75 hours after I^{131} administration. This is due to the fact that euthyroid individuals need this period of time to show a rise of I^{131} protein-bound iodine after a test dose. After successful I^{131} therapy, 2 patients previously having red cell-plasma ratios consistent with hyperthyroidism gave ratios which fell in the normal group when repeat test doses of I^{131} were administered. Further information concerning the I^{131} content of red cells, plasma and thyroid at various time periods in single patients which were typical examples of hyperthyroidism, euthyroidism, and hypothyroidism, is shown in Fig. 2. Such data were obtained on all of the patients described here in order to calculate their ratios.

In hyperthyroidism, I^{131} rapidly disappeared from the plasma reaching a minimum in the case shown at 8 hours after its administration. The initial drop in plasma concentration is due to accumulation by the thyroid and by excretion. Following this, a rise in plasma iodine was observed which was related to the I^{131} administered and represents protein-bound iodine in part. The curve of red cell activity also shows a drop at first but does not show a rise until several hours after the plasma begins to rise. This rise suggests that the lag between the plasma concentration and the red cell concentration represents the time needed for the catabolism of the labeled hormone, after which the iodine returns to the plasma as inorganic iodine and is capable of diffusing into the red cell. The hyperthyroid curves shown in Fig. II, depict thyroid metabolism in the exaggerated state. Normal thyroid metabolism as illustrated in Fig. 2 shows a less rapid disappearance of I^{131} from both red cells and plasma than in the hypothyroid. In addition, no increase in I^{131} activity of red cells or plasma was observed in the normals and hypothyroids during the time period for which they were studied. Iodine was lost at a decreasing rate from the red cells and plasma with the passage of time. This change in rate has also been noted by Myant *et al.*(6).

The fate of I^{131} with respect to red cells and plasma of a clinically myxedematous patient is also shown in Fig. 2. It may be noted from the curves that the I^{131} concentration of both red cells and serum fall off at what is practically an exponential rate and are parallel throughout their course. These curves indicate the excretion of iodide ion as would be expected in the absence of a functioning thyroid. No hormone is formed and the

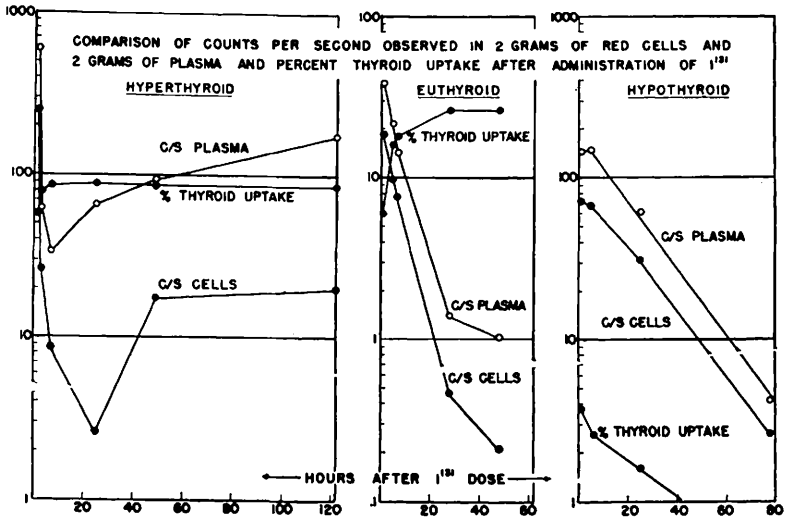

FIG. 2.
Distribution of I131 in thyroid and blood of patients which were hyperthyroid, euthyroid, and hypothyroid following oral administration.

relative concentrations of iodine in red cells and plasma is related to their essential iodide space. As was previously described, the ratio was found to be about 0.5 throughout the time of study.

In conclusion, we believe that, due to its simplicity, this method has more to offer than external measurements of I^{131} uptake by the thyroid or determination of I^{131} urinary output, in the diagnosis of thyroid disease. The red cell-plasma relationship illustrates not only the disappearance of inorganic iodine from the blood stream but its reappearance as protein-bound iodine as well.

Summary. 1. The ratio of red cell space for inorganic iodine to that of plasma was found to be about 0.5, in both *in vitro* and *in vivo* studies. 2. The initial red cell-plasma ratio was the same in hyperthyroid, euthyroid, and hypothyroid patients immediately after the oral administration of a test dose of I^{131}. 3. The ratio dropped as the inorganic iodine test dose was incorporated into larger molecules by the thyroid and returned to the blood stream. 4. The drop in red cell-plasma ratio was due to the relative impermeability of the red cell to large iodine-containing molecules. 5. The rate of drop of the red cell-plasma ratio is an expression of thyroid activity.

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