necrosis due to cystine deficiency in young rats was about the same as that observed in older rats but the young rats developed such lesions in 1/3 the time required in older rats. Many rats of all ages have been observed to die of cystine deficiency with no detectable histological abnormalities in any of 19 organs examined. Young rats, fed a diet deficient in both choline and cystine live somewhat longer than simply cystine deficient animals but show no hepatic damage. Older rats on such rations develop livers which are necrotic, fibrotic, and fatty despite a loss of as much as two-thirds of their initial body weight during the experimental period.

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Blood Levels of I-131 after Tracer Doses in the Diagnosis of Hyperthyroidism. (18269)

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Up to the present time, all methods employing radioactive iodine in the diagnosis of thyroid function have depended upon the well established fact that the hyperactive thyroid gland selectively concentrates and fixes larger amounts of I-131 than does the normal organ. This phenomenon has been measured in 2 ways; first by direct counting over the gland with a suitably arranged Geiger-Müller tube and second, by measuring the excretion in the urine during a fixed period after the administration of the tagged iodine. Both of these methods leave much to be desired so far as results consistent with the clinical state of the patient are concerned. There is a definite, often considerable, area of overlap between the normals and the hyperthyroids. In an attempt to circumvent these difficulties some observers have attempted to study the blood levels of I-131 after tracer doses and have sought additional information by fractionating the circulating I-131 into its inorganic and protein-bound fractions. These studies have again showed a considerable overlap between the normals and the hyperthyroids and have not given diagnostic information as clear-cut as might be desired. It occurred to us that a new approach might be fruitful. If one attempted to measure *secretion* of the thyroid hormone from the gland into the circulation rather than *fixation* of iodine by the gland more conclusive results might be attained. This has a physiological basis because it is the increased discharge of hormone from the thyroid gland rather than the increased avidity for iodine which accounts for the clinical phenomena of disturbed thyroid function.

Studies reported to date have usually been extended over a period of only 24 hours because it was known that fixation of iodine in the gland is quite complete in that period of time. If, however, one carries the observations over a longer period of time and studies blood levels of I-131, the changes due to fixation in the gland become less significant and changes due to hormone *secretion* dominate the findings. Although there is a difference between the amount of I-131 fixed in the glands of euthyroid and hyperthyroid subjects, these differences are not marked enough for a clear differentiation of the 2 groups of patients. However, at the end of 48, 72 or 96 hours after the administration of the tracer dose of I-131 there is a very marked difference in the level of I-131 in the blood and this difference has diagnostic value. The determination of the radioactivity of 1 cc of blood beyond 24 hours after safe tracer doses of I-131 could not be performed until counters of high sensitivity, such as the flow counters, became available.

Selection of clinical material. A consecutive series of 24 normal and 30 hyperthyroid subjects was selected from the wards of The Mount Sinai Hospital. The normal patients

were males and females on the wards of the Medical Services. They were selected because they presented no clinical or laboratory evidences of thyroid disease and their basal metabolic rates were within normal limits in all instances. The hyperthyroid patients all were undoubted clinical examples of this disease. The classical symptomatology of goitre, tachycardia, tremor and increased basal metabolic rate was present in every case. Classic eye signs were frequently present. In many instances the clinical diagnosis was confirmed by the presence of an elevated protein-bound plasma iodine level. At least 3 experienced clinicians agreed on the classification and no equivocal cases were included in this study. In the hyperthyroid patients the basal metabolic rates varied between + 20 and + 80%. In order to determine the effect of impaired renal function on the observations to be reported, a group of euthyroid patients with definite renal insufficiency was also studied. The basal metabolic rate of these patients was normal.

Methods. Carrier-free I-131* was administered intramuscularly in doses of 100 microcuries. Preliminary observations showed that, although the oral route could be used, less consistent results were obtained by this method. Intravenous administration had no advantage over the technically simpler intramuscular route. Blood was drawn by venupuncture at regular intervals. A small amount (1 mg) of potassium iodide as carrier and a drop of 10% sodium hydroxide were added to each blood sample. For the counting of whole blood or plasma, 1 cc was pipetted into a suitable planchette, dried and counted in a Q-gas counter (Model D 46, Nuclear Instrument and Chemical Corporation, Chicago). Protein-bound I-131 was determined by precipitating 1 cc plasma with trichloracetic acid, centrifuging and washing the precipitate 3 times with 15 cc portions of the acid. The final washed precipitate was dissolved in alkali and an aliquot representing 0.5 cc of the original plasma was pipetted into a planchette, dried and counted as before. Control studies showed that this procedure quantitatively removes larger amounts of inorganic I-131 than could be encountered in this study. Corrections were made for resolving time, background and decay to the beginning of the experiment. A dried sample of an aqueous solution of radioactive sodium iodide gave an average of 32,000 counts per second per microcurie with the equipment used.

TABLE I. Counts per Second Due to Protein-bound I-131 per cc of Plasma in Normals and Hyperthyroids After 100 Microcuries I-131 Intramuscularly.

Hr after inj.	4	24	48	72	96
			Euthyroids		
Avg	1.6	1.2	1.4	1.6	1.4
Range	0.6-3	1.0-2	0.4-1.8	1.0-2	0.8-2.2
			Hyperthyroids		
Avg	3.0	14.4	22.4	20.6	22.0
Range	1.0-4	2.6-73	5.6-83.4	5.4-73.6	6.0-77.8

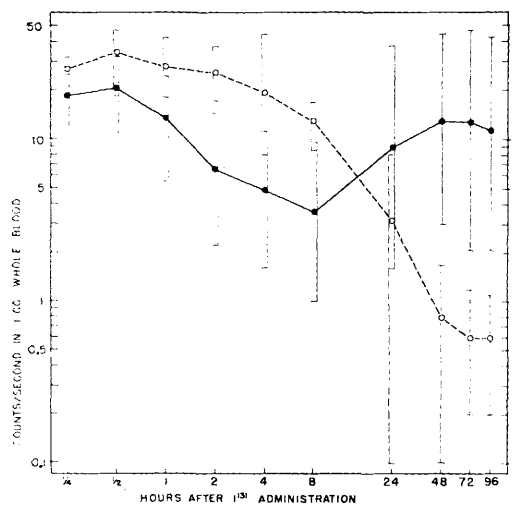

FIG. 1.

Activity in whole blood after 100 microcuries I-131 without carrier; administered intramuscularly. Dots and solid line: hyperthyroids (30 cases). Circles and dotted line: euthyroids (24 cases). Counts are net counts above background and calculated for decay to time of administration. The range of observed values is indicated for each time period. Ten counts per second is equivalent to .001 microcuries I-131.

Results. The results of these observations are presented in one figure and 2 tables.

In Fig. 1 where whole blood was counted it will be seen that at 48 hours and beyond there is a sharp separation of the euthyroid and hyperthyroid patients, in fact, there is

* The I-131 was supplied on allocation by the U. S. Atomic Energy Commission.

TABLE II. Counts per Second per cc of (a) Whole Blood, (b) Plasma, and (c) Protein-bound Plasma I-131, After 100 Microcuries I-131 Intramuscularly in Euthyroid Patients with Impaired Renal Function. Seven cases.

Hr after inj.	4	24	48	72	96
(a) Whole blood					
Avg	26.8	17.3	14.0	7.4	5.0
Range	12.6-44.9	5.1-37.0	3.8-27.2	2.9-20.2	0.2-2.6
(b) Plasma					
Avg	53.0	32.4	27.2	13.1	8.7
Range	23.1-81.2	13.2-71.7	6.4-48.4	6.1-31.9	0.6-26.3
(c) Plasma protein-bound					
Avg	1.4	1.4	1.6	0.8	1.6
Range	1.0-1.8	1.0-1.8	1.0-2.2	0.6-1.4	0.8-2.0

no overlap between the *highest* counts in the euthyroids and the *lowest* counts in the hyperthyroids. It is, of course, not unlikely that some overlap will be encountered as the number of cases studied is increased.

In Table I data on the same patients are presented showing the activity of the plasma due to protein-bound I-131. From these values it is apparent that the high counts found in the whole blood of the hyperthyroid patients at 48 hours and beyond is due to the presence in the plasma of relatively large amounts of protein-bound radioactive iodine; that is, iodine which has been incorporated by the thyroid gland into a protein complex and returned to the circulation as the hormone. A study of these values shows that the thyroid gland in hyperthyroidism produces hormone at a rate some ten times that of the normal gland if we assume that destruction of the hormone proceeds at a constant rate. It is generally accepted that the red blood cells are free of protein-bound iodine.

Table II shows the values obtained in a series of seven euthyroid patients who had renal insufficiency. In these, as in all other cases, the level of activity of the blood at any moment is the net result of several mechanisms at work simultaneously. These are:

1. The transport of the absorbed inorganic I-131
2. The removal of inorganic I-131 by the thyroid from the circulation
3. The excretion of inorganic I-131 by the kidney
4. The return to the circulation of protein-bound I-131 by the thyroid.

Factors 2 and 3 would tend to decrease the radioactivity of the blood while factor 4 would increase it. Factor 1 can be assumed to be relatively constant. It is a well accepted fact that with the impaired renal function the ability of the kidney to excrete iodides is decreased. Therefore, the total radioactivity of the blood should be increased under these circumstances if all the other factors remained constant. The fact that there is no increase in protein-bound I-131 shows that the high values obtained in euthyroid patients with renal disease is due entirely to the continued presence in the circulation of abnormal amounts of inorganic I-131 which the diseased kidney has been unable to clear —a situation quite different from that which exists in hyperthyroidism where the increased radioactivity of the blood is due to the presence of large amounts of protein-bound I-131 which have been returned to the circulation by the overactive thyroid gland. It can, therefore, be stated that a low count at 48 hours or beyond is diagnostic of the euthyroid state. A high count may be the result of thyroid hyperfunction or of delayed excretion due to renal (or "prerenal") disease. In the former the activity is due to protein-bound I-131 and in the latter to inorganic I-131. This distinction can be made by the methods described.

Summary. By prolonging the study of blood levels after tracer doses of I-131 to 96 hours we have been able to measure secretion of thyroid hormone into the circulation rather than concentration and fixation by the thyroid gland. This appears to be the best method presently available for clinical use

in the diagnosis of hyperthyroidism. A single blood or plasma sample of one cubic centimeter gives diagnostic values when counted after air drying. The determination of the protein-bound fraction only very rarely gives additional information but can be used to exclude spurious high levels as are occasionally found in cases of renal insufficiency where excretion of the circulating inorganic I-131 is delayed.

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A New Method for the Production of Experimental Bacterial Endocarditis. (18270)

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It was shown previously(1) that discontinuous exposure of rats to simulated high altitudes of 25,000 feet may produce fibrinous vegetations and thickening of the heart valves. The vegetations, except for absence of infection, were essentially similar to those found in human bacterial endocarditis. Because of this similarity and because bacterial endocarditis in man is confined chiefly to previously damaged valves, it was considered possible that rats having heart valves damaged by exposure to altitude would be particularly suitable for the study of experimental bacterial endocarditis.

The need for further investigation of endocarditis is indicated by reports(2-5) emphasizing the difficulties of treatment, the frequent recurrences, and the relatively high mortality (about 30%). Bacterial endocarditis has been produced with varying success in animals, chiefly rabbits, by massive intravenous injections of certain strains of *Streptococcus viridans* and other microorganisms(6-8). However, since the therapeutic response of bacterial endocarditis in man often varies widely with the specific strain of the causative organism, it seemed desirable to find a more suitable laboratory animal that could be infected with greater ease and certainly with a wide variety of organisms isolated from human cases of bacterial endocarditis. As will be shown here, rats exposed to altitude seem to meet these qualifications. Moreover, our findings, indicating that resistance to disease may be lowered by exposure to high altitudes, may be of interest also in aviation medicine and to those concerned with the health of populations living at very high altitudes.

Materials and methods. In a series of 100 consecutive cases of subacute bacterial endocarditis, Niven and White(9) isolated *Streptococcus mitis* in 45 patients, *S. sanguis* in 42, *S. bovis* in 12, *S. faecalis* in 5, and various other organisms in 9 patients. In our study 5 such strains of streptococci were used. Four were isolated from cases of subacute bacterial endocarditis (*S. mitis* JH-26, *S. sanguis* P25 Type 1 and 2, and *S. bovis* P-20*, and *S. mitis* Strain N†) and the fifth was obtained from an infection of the urinary tract (*S.*

1. Highman, B., and Altland, P. D., *Arch. Path.*, 1949, v48, 503.
2. McCoy, J. T. and Meyer, O. O., *Wisc. M. J.*, 1948, v47, 671.
3. Wagner, B. W., *Am. J. M. Sc.*, 1948, v215, 84.
4. Zeller, W. W., Hirsch, H. L., Dowling, H. F., Hussey, H. H., and Robinson, J. A., *M. Ann. District of Columbia*, 1948, v17, 21.
5. Henry, L., *Postgraduate Medicine*, 1950, v7, 366.
6. Welch, H., Murdock, T. P., and Ferguson, J. A., *J. Lab. and Clin. Med.*, 1936, v21, 1264.
7. MacNeal, W. J., Spence, M. J., and Wasseen, M., PROC. SOC. EXP. BIOL. AND MED., 1939, v40, 473.
8. MacNeal, W. J., Blevins, A., Pacis, M. R. and Slavkin, A. E., *Am. J. Path.*, 1945, v21, 255.
9. Niven, C. F., and White, J. C., *J. Bact.*, 1946, v51, 790