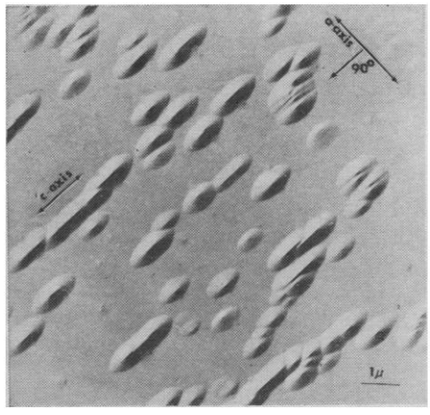

Fig. 3. Electron micrograph of a single crystal of TiB_2 annealed at 2300°C, showing etch pits on the $(10\bar{1}0)$ plane.

etch pits had the shape of triangles or hexagons on the basal or (0001) plane. Elliptical spiral etch pits (long axis parallel to the *c*-axis) were observed on the $(10\bar{1}0)$ plane. It was determined from the $(10\bar{1}0)$ plane that etch pits produced on $\{10\bar{1}0\}$ planes of annealed single crystals (2300°C) were at an angle of 30 degrees to the Burgers vector, which is parallel to the dislocation line (Fig. 3) (*7*). This means that the slip direction must be of the $<11\bar{2}0>$ type. The findings were then correlated with the results of the high-pressure experiments on polycrystalline TiB_2. Since we knew the plane orientations on the single crystals and could compare the slip traces of the single and polycrystals, we could also determine the planes of the corresponding polycrystalline grains. The slip occurring in both single and polycrystalline TiB_2 was found to be the same, namely, of the type $\{10\bar{1}0\}$ $<11\bar{2}0>$.

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Metabolism of Iodine-131–Labeled Thyroxine-Binding Prealbumin in Man

Abstract. *The metabolism of ^{131}I-labeled thyroxine-binding prealbumin was studied in four normal subjects and in five hospitalized patients with nonthyroidal disease. The average half-time of total-body thyroxine-binding prealbumin, calculated on the basis of a two-compartmental model, was 1.90 days in normal subjects. Increased fractional degradation or diminished synthesis, or both, contributed to the depression of thyroxine-binding prealbumin in the serum of patients with nonthyroidal disorders.*

Thyroxine-binding prealbumin (TBPA) first described by Ingbar (*1*), is recognized as one of three serum proteins taking part in the peripheral transport of thyroxine (*2*). Experiments in our laboratory have indicated that this protein is important in regulating the concentration of free thyroxine in the serum (*3, 4*). In patients with systemic nonthyroidal illness a diminished concentration of TBPA frequently results in an increase of free thyroxine in the serum. (*3*). Furthermore, within 2 to 4 days after a major surgical procedure serum TBPA falls to approximately one-half of the preoperative value with a concomitant rise in the concentration of free thyroxine (*4*). The free thyroxine concentration is considered a better index of the overall thyroidal status of an individual than is the concentration of total circulating hormone (*5, 6*).

We have isolated TBPA in an immunologically pure form (*7*). The protein has a molecular weight of 73,000, a sedimentation coefficient of 4.58×10^{-13} sec, and a diffusion constant of 5.93×10^{-7} cm²/sec. The protein is identical with prealbumin-1 (PA-1) separated by starch-gel electrophoresis. It has a single thyroxine-binding site per molecule, and like the prealbumin preparation of Schultze, Schonenberger, and Schwick (*8*), it is rich in tryptophan. We now describe results of metabolic studies in four normal male subjects and five hospitalized patients with varying concentrations of serum TBPA. There was rapid turnover of TBPA in comparison with that of other plasma proteins. Certain aspects of these studies have been reported (*9, 10*).

The TBPA was isolated from normal serum by means of cellulose-column electrophoresis and concentrated on diethylaminoethyl cellulose (DEAE) columns (*7*). In order to render the protein safe for administration to human subjects, the preparation was pasteurized (60°C, 10 hr) to inactivate any possible hepatitis virus. This procedure did not change the electrophoretic behavior of TBPA or alter the maximum thyroxine-binding capacity per milligram of protein. Similarly treated albumin has been generally used in turnover studies of radio-iodinated albumin in man. In a single study in which heat treatment of albumin was omitted (*11*) the overall metabolism of the iodinated protein in normal subjects was similar to that of human serum albumin which had been lightly iodinated and heated.

The initial studies were carried out on a single lot of TBPA iodinated (method I) at Abbott Laboratories, Oak Ridge, Tennessee (*12*). One milliliter of a solution of 10 mg TBPA in 0.1*M* phosphate buffer, *p*H 7.4, was mixed with $K^{131}I$ and nonradioactive KI. The iodide was oxidized to iodine by dilute hypochlorite solution. Excess iodide was removed by passage through an ion-exchange column and subsequent dialysis against isotonic saline. The iodinated TBPA had an initial specific activity of 0.744 mc/mg and contained approximately 1.4 atoms of iodine per molecule. In order to minimize radiation damage, the final radio-iodinated product was immediately mixed with a 1-percent solution of human serum albumin (Albumisol; Merck, Sharp, and Dohme) in isotonic saline. When subjected to vertical starch-gel electrophoresis, 90 percent of radioactivity migrated as a sharp symmetrical peak anodal to serum albumin, but approximately 10 percent of the radioactivity was associated with a peak in the albumin area. When added to serum, ^{131}I-TBPA migrated with the leading edge of PA-1. Less than 2 percent of the radioactivity was dialyzable, suggesting neglible contamination with ^{131}I-iodide. The final material was sterilized by passage through a 0.22-μ Millipore filter. This preparation was used in studies on JO, MS, JS, HB, MK, and YF.

It became more convenient to iodinate TBPA in our laboratory as needed for individual studies (method II) by the technique of Greenwood, Hunter,

and Glover (*13*). Approximately 100 μg of a separate lot of pasteurized TBPA was iodinated on three occasions with about 2 mc of carrier-free $Na^{131}I$. The final specific activity was approximately 4.5 mc/mg. This material had similar characteristics on starch-gel electrophoresis. Approximately 8 percent of the radioactivity was associated with a peak in the albumin area, and this contaminant was removed by a preparative starch gel electrophoresis with continuous elution (*14*), the forerunning fraction being reconcentrated by DEAE columns (*6*). Starch-gel electrophoresis of the final product revealed that more than 99 percent of the radioactivity was associated with the prealbumin peak. The ^{131}I-TBPA was mixed with a 1 percent solution of human albumin and sterilized by filtration through a Millipore filter. The three iodinated preparations made by this method were used in the studies on GB, GP, and PR, respectively.

Metabolic studies were carried out in four normal ambulatory male subjects and five hospitalized patients with varying concentrations of TBPA in the serum. During the study, all individuals received five drops of Lugol's solution every 8 hours in order to minimize the thyroidal accumulation of radioactive iodine. Five to 17 μc of ^{131}I-TBPA, dissolved in a volume of 5 to 15 ml, were administered intravenously from weighed syringes. Radioactivity of plasma samples and daily urinary excretion of ^{131}I were measured for 8 to 10 days after injection of the labeled protein. Fecal excretion of isotope was also measured in all subjects and patients except MK. Precipitation of plasma proteins with 20 percent trichloroacetic acid (TCA) indicated that more than 95 percent of the circulating radioactivity was protein-bound. Precipitation with TCA of urine to which nonradioactive plasma had been added showed that more than 95 percent of the urinary radioactivity was not bound to protein, and was presumably in the form of ^{131}I-labeled iodide. The serum TBPA was determined by the densitometric analysis of starch-gel electrophoretic patterns stained for protein (*4*). The intensity of the PA-1 band of the test serum was compared to the intensity of a reference serum in which the absolute concentration of TBPA had previously been obtained by extrapolation after the addition of known quantities of purified TBPA (*7*).

We analyzed the data by the graphic method of Pearson, Veall, and Vetter (*15*) who assumed a two-compartmental distribution of the plasma protein. The data on turnover in normal subject GB, studied with ^{131}I-TBPA prepared by method II, were quite similar to those obtained in the three other normal subjects studied with ^{131}I-TBPA prepared by method I (Fig. 1, Table 1). The removal of contaminating ^{131}I-protein migrating in the albumin region in method II may have been responsible for a somewhat more linear terminal portion of the curve of decay of ^{131}I in the plasma. This effect, however, was not marked, and it appeared justifiable to combine data obtained with iodinated TBPA prepared by either method I or II. The results of studies of turnover in normal subjects and hospitalized patients are summarized in Table 1.

It will be useful to compare the results in normal subjects to similar data on the turnover of ^{131}I-albumin in normal volunteers (*11*). The distribution of TBPA between vascular and extravascular compartments (49:51) is roughly similar to that found for serum albumin (46:54). As in the case of ^{131}I-albumin, the isotope is excreted primarily in the urine. Less than 3 percent of the label originating from the ^{131}I-TBPA degraded per day was excreted in the stool. What distinguishes

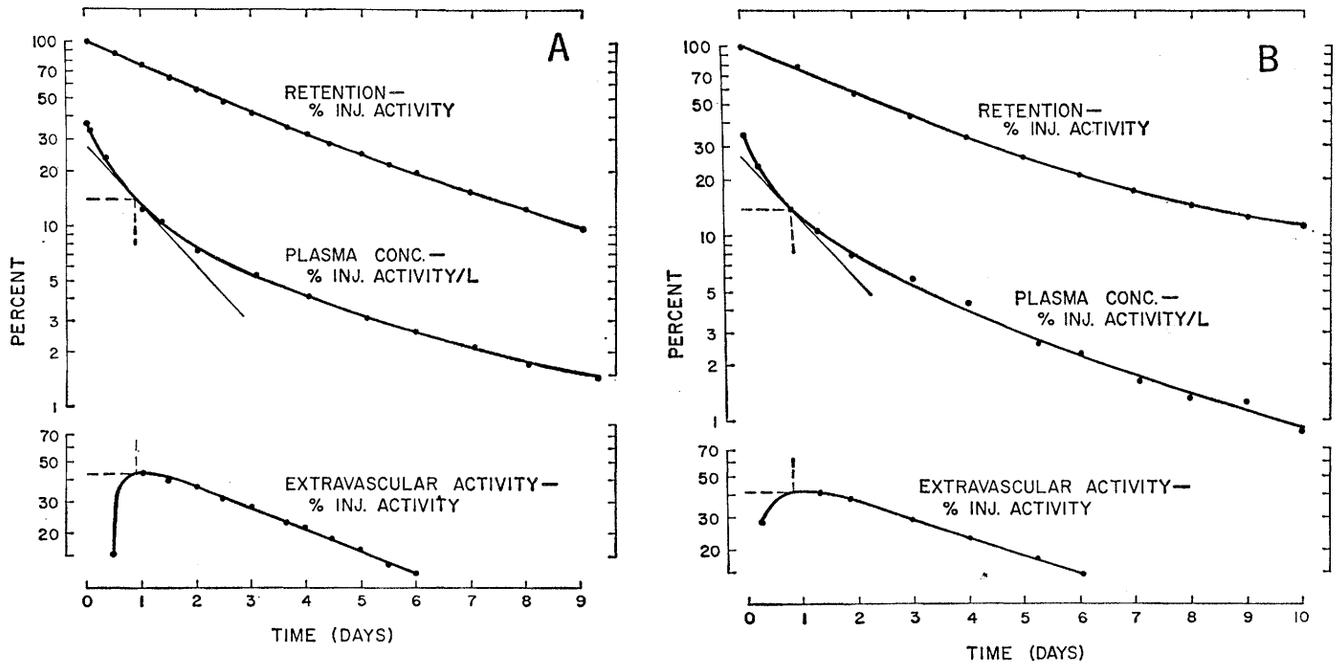

Fig. 1. Turnover studies in two normal subjects. (*A*) MS, studied with ^{131}I-TBPA prepared by method I. (*B*) GB, studied with ^{131}I-TBPA prepared by method II. The slope of the thin solid line is determined from the fractional degradation of the ^{131}I-TBPA in the plasma compartment and is drawn tangentially to the plasma radioactivity curve in order to determine the point of isotopic equilibrium between vascular and extravascular compartments (*15*). This point is designated by the broken lines. Notice the close correspondence of this point to the point at which a maximum quantity of radioactivity is present in the extravascular compartment, in agreement with theoretical expectations of a two-compartmental system (*15*). *INJ.*, injected; *L*, liter.

Table 1. Summary of turnover studies of TBPA. Diagnoses: HB, pulmonary tuberculosis; MK, hepatic cirrhosis; YF, carcinoma of stomach; PR, carcinoma of lung; GP, congestive heart failure. Abbreviations: *K*, fractional degradation per day of total body TBPA (*15*); *I:E*, intravascular to extravascular distribution; Fec., fecal degradation; Degrad., degradation.

Subject	Age	Sex	Wt. (kg)	Serum conc. (mg/100 ml)	Total pool (mg)	*I* : *E*	*K*	Fec. (mg kg^{-1} day^{-1})	Degrad. mg kg^{-1} day^{-1}
Normal subjects									
JS	32	M	66.3	23.0	1500	51 : 49	0.331	0.20	7.48
MS	29	M	84.0	36.4	2070	47 : 53	.359	.19	8.87
JO	36	M	82.7	35.4	2285	52 : 48	.395	.31	11.0
GB	31	M	72.7	40.1	2291	48 : 52	.367	.43	11.6
Mean:				33.7	2036	49 : 51	.363	.28	9.74
Patients hospitalized with nonthyroidal disease									
HB	56	M	57.2	17.1	1100	49 : 51	.548	0.31	10.5
MK	60	M	95.4	7.7	675	47 : 53	.389		2.76
YF	74	F	57.2	14.3	546	60 : 40	.587	.88	5.61
PR	49	M	55.0	7.0	367	57 : 43	.556	.33	3.71
GP	62	M	64.0	23.0	705	68 : 32	.879	.38	9.54

TBPA from serum albumin from a kinetic standpoint is the rapid fractional degradation of the protein. On the average, 36.3 percent of total body TBPA is degraded per day (*16*); this corresponds to a mean half-life of 1.90 days. When the data of Beeken *et al.* (*11*) are expressed in a similar fashion, an average of 5.44 percent of total body serum albumin is degraded per day, yielding a calculated half-time of 12.7 days. Although the serum TBPA concentration is less than 1/100 that of serum albumin, the more rapid turnover of TBPA results in a total daily degradation of TBPA (9.74 mg/kg) only 1/20 that of serum albumin (196 mg/kg).

Of the hospitalized patients with nonthyroidal disease, four out of the five had significantly lowered concentrations of circulating TBPA; the fifth patient (GP) had a borderline value. An increased fractional rate of TBPA degradation was observed in all patients except MK. The enhanced loss of radioactivity in the feces of patient YF contributed to but did not entirely account for the increased fractional degradation. Since no significant changes in serum TBPA levels were observed in the course of each study the patients can be considered to have been in a steady state with the rate of synthesis equal to the rate of degradation. Thus, three patients (MK, YF, and PR) had a diminished rate of synthesis as expressed in mg kg^{-1} day^{-1}. The diminution of synthesis was especially marked in the two patients with the lowest serum TBPA concentration (MK, PR). Two mechanisms, therefore, contribute to the depression of serum TBPA in patients with nonthyroidal disease: diminished synthesis and increased fractional degradation. The significance of the increased ratio of vascular to extravascular TBPA observed in two patients (YF and GP) is unknown. Further studies are indicated to elucidate the abnormalities of TBPA metabolism in patients with nonthyroidal disease.

The finding of a rapid turnover of iodinated protein always raises the possibility that the accelerated metabolism is due to denaturation of the protein, during either its preparation or iodination. The following considerations, however, tend to negate this explanation of the short half-life observed. (i) Results of several studies with two preparations of TBPA isolated by gentle techniques and lightly radio-iodinated by two different methods were substantially the same. (ii) There was no evidence on electrophoresis of alteration in protein structure. (iii) A relatively constant ratio of daily excretion of ^{131}I to its concentration in plasma was observed throughout the study. (iv) ^{131}I-Albumin, isolated and iodinated (*7*) by techniques identical to those employed in the preparation of ^{131}I-TBPA, was injected into one normal subject. The kinetic characteristics of the turnover of this material was well within the normal range of values reported by Beeken *et al.* (*11*) for ^{131}I-albumin. (v) The short half-life of TBPA accords with the rapid disappearance of the TBPA after surgical procedures. In other studies we have shown that after surgery there is an almost complete cessation of TBPA synthesis (*10*).

The limitations in the use of ^{131}I-labeled proteins for metabolic studies are generally recognized, and it is quite possible that minor alterations in TBPA produced during the process of iodination might make ^{131}I-TBPA a less than perfect tracer of the endogenous protein. Furthermore, the assumption of a two-compartmental model, though generally consistent with our observed results, may be an oversimplification of the situation in vivo. For these reasons, the values derived in this study must be regarded as approximations. Our data, however, establish beyond reasonable doubt that TBPA has a rapid metabolic turnover in comparison to that of other plasma proteins.

Since this report was submitted independent studies by Socolow *et al.* on the turnover of ^{131}I-TBPA in normal subjects and chronically ill patients have been reported (*17*). When a similar method of compartmental analysis is applied to their results on normal subjects their results and ours are in substantial agreement. In the three patients with nonthyroidal disease studied by Socolow *et al.*, diminished synthesis was found to be the only factor responsible for the reduction of circulating TBPA.

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$$K = \left(\frac{\text{Plasma pool of TBPA}}{\text{Total body pool of TBPA}} \right) \times k$$

where k is the experimentally determined fraction of plasma radioactivity excreted per day (*15*).
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Immunization against Rauscher Mouse Leukemia with Tissue Culture Material

Abstract. *Long-term monolayer cultures of mouse thymus and spleen cells, chronically infected with Rauscher virus, showed only a very attenuated leukemia-producing capacity. Material from these cultures prepared from normal BALB/c mouse tissue was used to immunize isologous mice against subsequent challenge with fully active Rauscher virus. The centrifuged supernatant of the culture had some immunizing properties. However, the most efficient protection was obtained when cell suspensions from these cultures were used in the form of a series of three intraperitoneal and intramuscular injections and one booster injection.*

Immunological reactions of the host in leukemias of the Friend and Rauscher types have been reported. Initially Friend (*1*) demonstrated that previous inoculations of susceptible mice with formalin-treated extracts from leukemic spleens could protect against challenge with the active virus. Fink and Rauscher (*2*) obtained similar results with the Rauscher virus. Specific new antigenic components in the Friend leukemia have been demonstrated by cytotoxic tests in vitro (*3*) and with the aid of immunofluorescence techniques (*4*).

The aim of the experiments reported here was to explore the possibility of protecting mice against leukemia by immunization with long-term infected cultures of normal lymphoid mouse cells; the cultures were latently infected with the Rauscher virus and showed an attenuated leukemia-producing capacity.

The original strain of Rauscher virus was given to us by Dr. Rauscher. The currently used virus stock was prepared from a centrifuged homogenate (10 percent in 0.153*M* potassium citrate) of spleens from diseased animals. Its titer, as determined by intraperitoneal inoculations of 1-month-old BALB/c mice, was of the order of 10^6 LD_{50} (lethal dose, 50 percent effective) per milliliter. Mice of the BALB/c strain, aged 1.5 to 2 months, were used uniformly throughout these experiments. The development of the Rauscher disease in the virus-inoculated animals was checked routinely by spleen palpation and hematological examination. Satisfactory agreement was established between the estimation of splenomegaly in living animals and the spleen weights as verified in killed mice. In general, palpation, scored from 0 to plus 4, corresponded to spleen weights of less than 0.3 g, 0.3 to 0.6 g, 0.6 to 1 g, 1 to 2 g, and more than 2 g.

The V5 cell line developed in vitro by Wright and Lasfargues (*5*) from mixed BALB/c spleen and thymus cultures was infected with the Rauscher virus and reproduced the virus actively for more than 80 passages, apparently without harm to the reticulum-type cells multiplying rapidly in these cultures. In our laboratory the V5 line was cultivated in NCTC 109 synthetic medium supplemented with 20 percent calf serum.

In November 1964, the supernatant of the V5 cultures, diluted 10 times or undiluted, was inoculated intraperitoneally at a dosage of 0.1 ml into 16 BALB/c mice, aged 1 to 2 months. No leukemias were observed in any of the inoculated animals. At the same time, cells from the cultures were inoculated intraperitoneally into five BALB/c mice of the same age, at dosages of 1 million cells per mouse. Again no symptoms of the Rauscher disease were noted in any of these animals.

Consequently, experiments were designed to check whether mice that received prior inoculation of the culture supernatant and then remained symptom free would resist challenge with the fully active Rauscher virus.

In two independent series, 0.1 ml quantities of the V5 culture supernatant were inoculated into 1-month-old BALB/c mice. About 23 or 27 days later the mice, all of which appeared to be healthy, were inoculated with the infectious stock virus in parallel with control animals (Table 1).

As can be seen from the table, half of the animals that had been inoculated with tissue culture supernatant were protected against the challenge with relatively high doses of virulent virus.

In a parallel series, 13 mice inoculated with only the culture supernatant remained apparently healthy during the 150-day observation period.

Since these results were indicative

Table 1. Results of inoculation (challenge) of Rauscher virus into control mice and mice that received prior treatment with V5 supernatant. The incidence of splenomegaly in each group is recorded out of the total number of mice in each group.

Days after challenge							Survival 150 days after challenge
5	9	13	19	22	26	32	
Control mice							
0/10	5/10	9/10	10/10	10/10	9/9	9/9	0/10
Mice with prior inoculation with culture supernatant							
0/14	3/14	6/14	7/14	7/14	7/14	7/14	7/14

Table 2. Results of immunization of BALB/c mice with supernatant and cells from V5 cultures. The material indicated was inoculated on days 1, 4, and 7 intramuscularly and intraperitoneally, and on day 22 intraperitoneally. Groups III, IV, and V were challenged with virulent Rauscher virus on day 28. Groups I and II were not challenged. CS, culture supernatant.

Mice		Material inoculated	Leukemic animals*/ total
Group	No.		
I	9	CS	3/9
II	10	CS and cells	0/10
III	10	Stock medium	8/10
IV	19	CS	9/19
V	19	CS and cells	0/19

* Observations at the 55th day after challenge based on spleen palpation and hematological examinations.