STUDIES ON THE MODE OF ACTION OF SYNTHETIC THYROPROTEIN

By

Wallace Friedberg

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology and Pharmacology

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Approved E. P. Reineke

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Studies were conducted with the view of increasing our knowledge of the mode of action of synthetic thyroprotein in the living body. The thyroprotein used was I-131 labelled iodinated casein. The first experiment was a comparative study of the distribution of iodinated casein and iodide in various tissues of the rat. Radioiodide and iodinated casein were administered, intravenously, to two groups of rats. One hundred minutes after the injections the animals were sacrificed and tissue samples were taken. Higher concentrations of iodinated casein than iodide were found in the liver, spleen, kidney, lung, stomach, and small intestine. The highest concentration was found in the liver, where it was more than 10 times that of the iodide. Slightly iodinated casein (1-2% iodine) was studied similarly and was found to concentrate in the same tissues which were shown to concentration iodinated casein. The slightly iodinated casein was not as concentrated in the liver, but more concentrated in the stomach and large intestine, than the iodinated casein.

The metabolic breakdown of intravenously administrated iodinated casein was studied with a view toward characterizing the metabolites and determining their relative concentrations

over a time series. The alkali washed butanol extract was considered to contain the thyroxine-like iodine. The free thyroxine-like iodine was that which could be extracted before hydrolysis of a tissue sample. Combined thyroxine-like iodine was that which was extractable after the free thyroxine-like iodine had already been extracted and the tissue sample hydrolyzed in 2N sodium hydroxide. The time intervals studied were 1½, 12, 24, and 72 hours. The results indicated that thyroxine-like iodine was hydrolyzed from the iodinated casein, in the liver, and released to the plasma. The amount of iodinated casein available to the liver and the plasma level of thyroxine-like iodine were found to be interdependent.

Orally administered iodinated casein was studied in rats in order to characterize the iodine absorbed into the plasma, and to determine the relationship between the time interval after oral administration of the thyroprotein and the concentration of thyroxine-like iodine in the plasma. The time intervals studied were 1½, 12, and 48 hours. More than 50 percent of the thyroxine-like iodine appearing in the plasma, at all time intervals after oral administration, was in the free butanol soluble form. However, the data suggest that small amounts of thyroxine-like iodine, in a combined form, are absorbed from the intestinal tract. The highest percent of combined thyroxine-like iodine was found at the first time interval (1½ hour). This may represent the intact protein or some large metabolite.

The influence of plasma iodinated casein on urine radioactivity was studied by measuring the decreases in plasma and urine radioactivity in the dog. Iodinated casein was found to be rapidly cleared from the plasma while the urine radioactivity showed a slow, but steady, decrease. A characterization of the radioactive material in the urine, 25 minutes after the intravenous administration of iodine-131 labelled iodinated casein revealed it to be undialyzable. The radioactivity in the urine may represent the intact iodinated protein or some large metabolite.

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INTRODUCTION

Investigations carried out by Reineke, et al (1943, '45, '48) and others (Frieden and Winzler, 1948) have shown that parentally administered iodinated casein exhibits the physiological properties of thyroxine. This biological activity has been attributed to thyroxine formed in the casein during its *in vitro* iodination. Of particular interest is the fact that the thyroxine in iodinated casein is in strong combination and hours of hydrolysis with strong acid or alkali is necessary for its liberation. In contrast, thyroxine normally found in the blood is only loosely bound to the serum proteins.

The question arises: Does the iodinated protein per se exert a biological effect or is it first metabolized with the release of thyroxine? Experiments were designed to trace the pathway of this protein-combined hormone, principally in the rat. The problem has been divided into the following classifications:

1. The distribution of iodinated casein in comparison with iodide in the rat.
2. The distribution of slightly iodinated casein in the rat.
3. The metabolism of iodinated casein in the rat.

4. The disappearance of iodinated casein from plasma and appearance of its metabolic product in the urine, in the dog.

The literature has been reviewed in order to trace the development of the techniques used in these experiments so that they may be properly evaluated. It also serves to summarize the available information related to the metabolism of iodinated protein. Specifically, the review will consider:

1. The formation of iodinated protein with high thyroidal activity.
2. The quantitative extraction of thyroxine from the hydrolysate of iodinated protein.
3. The metabolism of iodinated protein.
4. Iodine-containing compounds in iodinated casein.

It is hoped that the experiments described in this paper will contribute toward an understanding of the biochemistry of iodinated protein.

REVIEW OF THE LITERATURE

Formation of Iodinated Protein with High Thyroidal Activity

Blum and Vaubel (1898) studied the conditions for iodinating albumin. They found that hydriodic acid, liberated when iodine combined with the protein, inhibited the reaction. By buffering the aqueous medium with sodium bicarbonate they were able to neutralize the acid as it was formed. When iodinated by this procedure the protein held about 7 percent iodine in firm combination. Present-day methods for the formation of iodinated proteins with high thyroidal activity are based on these early findings.

Abelin (1934) clearly demonstrated that the acid-insoluble fraction of the hydrolysate of iodinated protein exhibits all the biological properties of the thyroid hormone.

Direct proof that thyroxine is present in iodinated protein hydrolysate was obtained by Ludwig and Von Mutzenbecker in 1939. These investigators isolated thyroxine from the hydrolysate of iodinated casein.

Reineke, et al (1942, '43, '45) carried out extensive investigations in an effort to produce iodinated casein with high thyroidal activity. They reported that incubation at elevated temperatures (60-70° C.) and the presence of small

amounts of oxides of manganese, as a catalyst, caused a significant increase in the biological potency of the material. The amount of iodine employed and the pH of the medium were also found to influence the biological activity of the thyroprotein. Courrier and coworkers (1949) contend that the incubation at elevated temperatures does not increase the thyroxine content but does increase the absorbability of the iodinated casein from the gastrointestinal tract.

The Quantitative Extraction of Thyroxine from the Hydrolysate of Iodinated Protein

Present-day assay methods involving a quantitative extraction of thyroxine have their origin in the work of Kendall (1915). The object of his investigations was to isolate, in pure form, one or more of the constituents of the thyroid gland with physiological activity. Carrying out a preliminary study of the nature of the iodine combination, he observed that suspended or dissolved desiccated thyroid lost less than 5 percent of its total iodine when dialyzed against running water. Following hydrolysis with sodium hydroxide in alcohol, 75 percent of the iodine was dialyzable; however, it still remained in organic combination. The hydrolysate could be separated into two fractions according to solubility: about 50 percent being acid soluble, and 50 percent acid insoluble. Kendall (1915) found the acid-insoluble fraction to be of an acidic nature, easily soluble

in dilute alkali or ammonia, and reprecipitated by any acid. Further purification of the acid-insoluble fraction was accomplished by extracting it with petroleum ether to remove fatty acids and sulfur. The resulting dark brown powder was effective in the treatment of myxedema; subnormal pulse and temperature were raised to normal, and metabolism was increased. Symptoms of hyperthyroidism could be excited by administering the preparation to normal dogs. Continuing his studies, Kendall (1919) hydrolyzed fresh thyroid glands in sodium hydroxide and obtained a clear alkaline filtrate containing almost all the iodine. Acidification of this solution resulted in the separation of a fine flocculent precipitate weighing 0.1 percent of the total weight of the fresh glands and containing about 26 percent of the total iodine. After further purification of this material he was successful in isolating thyroxine. Harington and Randall (1929) devised a chemical assay for thyroxine in the thyroid, based on the quantity of acid-insoluble iodine in the hydrolysate of the gland. Specifically, thyroid material was hydrolyzed for 4 hours in N/1 sodium hydroxide and the filtrate adjusted to pH 5 with 50 percent sulfuric acid. The acid-insoluble iodine was considered to be thyroxine iodine.

While the method of Harington and Randall (1929) was simple technically, it was shown by Leland and Foster (1932) that only about half of the acid-insoluble iodine goes to make up thyroxine. The latter investigators based their

method on the extraction of thyroxine with butanol after alkaline hydrolysis of the gland. Desiccated thyroid was hydrolyzed for 18 hours with 2N sodium hydroxide and the cooled hydrolysate was extracted with butanol. The alcoholic extract was further purified by shaking it with N/1 sodium hydroxide. In a series of 52 human thyroids the mean thyroxine iodine was 25 percent of the total iodine. Recovery experiments indicated that the amount of thyroxine destroyed during the alkaline hydrolysis was not more than 15 percent of the total thyroxine.

With a view toward increasing the accuracy of the technique of Leland et al, Blau (1933) made revisions in the method. He found that, by shaking an acid suspension of thyroxine with butanol, usually 100 percent of the suspended thyroxine passed into the organic phase in a single extraction. Therefore, he acidified the alkaline hydrolysate of thyroid gland, extracted it with butanol, and then washed the extract with an alkaline solution consisting of 5 percent sodium carbonate in 4N sodium hydroxide. With this modified procedure, Blau was able to obtain thyroxine values in thyroid material which were 10 to 20 percent higher than those obtained by the earlier method of Leland and Foster (1932).

Further investigations led Blau (1935) to conclude that the chief shortcoming of the Leland and Foster (1932) technique was the amount of thyroxine destroyed during the hydrolysis. After boiling crystalline thyroxine for 18

hours with dried and defatted beef muscle in 2N sodium hydroxide, only about 85 percent could be extracted from the acidified solution. Following the lead of Harington (1926), who used barium hydroxide as a hydrolytic agent in the isolation of thyroxine from thyroid gland, Blau boiled the thyroid material with an 8 percent solution for 6 hours. When compared with an equivalent concentration of sodium hydroxide he found that the barium hydroxide accelerated the rate of hydrolysis and rendered the thyroxine more readily extractable. The loss of thyroxine was only about 10 percent.

Reineke et al (1945) were interested in a chemical procedure for assaying iodinated casein with high thyroidal activity. They found that the apparent thyroxine content according to Blau's (1935) method, was considerably higher than a biological assay based on the metabolic stimulation of guinea pigs. It seemed to them that the high chemical analysis might be attributed to incomplete hydrolysis of iodinated non-thyroxine groups in peptide combination with the thyroxine. They carried out the hydrolysis in 40 percent barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$) for 20 hours and used a butanol extraction technique, modified, but similar in principle to Blau's. The resulting thyroxine values agreed closely with those determined by the metabolic stimulation of guinea pigs. Furthermore, butanol extracts of the iodinated casein, prepared the same as for the chemical technique, produced a metabolic effect equivalent to the thyroxine

content determined chemically. Taurog and Chaikoff (1946) modified Blau's procedure by hydrolyzing the thyroid in 2N sodium hydroxide for 15 hours. While the sodium hydroxide caused greater destruction of thyroxine than did barium hydroxide, thyroxine values were less variable with the former alkali.

Recent investigations reveal several shortcomings of the butanol extraction technique for thyroxine determinations. Hird and Trikojus (1948) reported the presence of several ninhydrin-positive materials in the butanol extract of iodinated casein hydrolysate, which they tentatively identified as thyroxine, triiodothyronine, and diiodothyronine. Their identification was based on rf values obtained by chromatographic analysis.

In 1949, Reineke et al applied the isotope dilution technique to the determination of thyroxine in iodinated casein. They reported thyroxine values which were about 25 percent of those obtained by the butanol extraction method (Reineke et al, 1945).

Friedberg and Reineke (1951, 1952) carried out chromatographic studies on the hydrolysate of iodine-131 labelled iodinated casein. Radioautograms of the chromatograms revealed the presence of at least three iodinated thyroxine-like compounds. After purifying the butanol extract with Blau's alkaline solution (1933), they found that the concentrations of these iodinated materials were not significantly

reduced. These thyroxine-like products would be included in the thyroxine fraction, as determined by the usual butanol extraction procedure.

The Metabolism of Iodinated Protein

The animal body responds to synthetic thyroprotein in a manner similar to its response to thyroxine. Investigations conducted by Käer (1934) demonstrated that iodinated protein exerts a thyroxine-like effect without the necessity of a preliminary treatment. She used a preparation of iodinated albumin which had previously been studied by Barkan and Leistner in 1929. The thyroprotein was fed, intact, to tadpoles, and the change in their size and development noted; artificial iodinated albumin was found to accelerate metamorphosis. Käer also found the iodinated albumin to be effective when fed to young guinea pigs; a decrease and, sometimes, stoppage of weight gain was observed.

Lerman and Salter (1939), conducting clinical studies, found iodinated blood serum protein to be effective for the relief of myxedema. The protein was iodinated in ammoniacal solution, according to the procedure of Wormall (1930). Four patients with athyreosis were treated and there was rapid and complete recovery with proper dosage; BMR increased, diuresis occurred, and blood cholesterol dropped. Assaying iodinated casein in tadpoles (1942), guinea pigs (1945), and sheep (1946), Reineke and Turner found that the parenterally

administered material exerted hormonal activity without any toxic side effects. In subsequent experiments carried out by Frieden and Winzler (1948) the goiter prevention method (Dempsey and Astwood, 1943) was employed to assay iodinated casein, anda parenteral route of administration was also used successfully.

Turner and Reineke (1946) investigated the relationship between the route of administration of iodinated casein and its utilization by ruminant animals (sheep). The criterion of thyroidal activity was the loss in body weight during a two-week period. Iodinated casein was found to be 20 times as effective when injected subcutaneously as when administered orally. They further reported that iodinated casein, when administered orally in dry form in a capsule, was nearly twice as effective as when suspended in a slightly alkaline or phosphate buffer solution and given as a drench. When iodinated casein was administered directly into the abomasum of sheep, in order to bypass the rumen, no increase in utilization was observed. Thus, the rumen was eliminated as a specific site of destruction of iodinated casein.

Reineke and Turner (1944) also studied the permeability of the mammary gland to iodinated casein and reported that biologically detectable amounts of the thyroid hormone were not found in the milk of lactating cows which had been fed massive doses of iodinated casein. The milk was tested for hormone activity by feeding it to guinea pigs, thyroidectomized goats, and human subjects.

Campbell et al (1950) conducted studies with iodinated casein labelled with radicactive iodine. When this material was fed to lactating ewes, the skimmed milk showed a considerable amount of radioactivity. About 20 percent was associated with the protein portion, the remainder being extractable with butanol. Thirty percent of the activity in the skimmed milk showed a similar solubility in alkaline butanol as thyroxine; it was, therefore, classified as the thyroxine fraction. When labelled iodinated casein was administered orally to rats, and the plasma was fractionated 10 hours later, 57 percent of the plasma was not extractable with butanol. Campbell et al (1950) suggested that this fraction may represent the iodinated protein per se, or some non-butanol soluble metabolite. When iodinated casein was injected into the jugular veins of sheep, 90 percent of the radioactivity was removed from the blood stream in 7 hours. As determined by radioactivity measurements of sheep blood, there was greater absorption of iodinated casein following oral administration than when the material was administered into the ventral sac of the rumen, or small intestine; there was practically no absorption from the cecum.

Iodination of a protein causes changes in its biological properties which apparently are not related to its thyroxine-like activity. Wormall (1930) observed an immunological characteristic which was specific for all iodinated proteins. The antigenically active moiety of heavily iodinated

proteins is their determinant diiodotyrosine group; the non-iodinated part acts only as a carrier. Thus, all heavily iodinated proteins have the same or a very similar antigenic specificity.

Another characteristic of heavily iodinated, as well as brominated, proteins is their rapid disappearance from the circulating plasma. Fine and Seligman (1943, 1944), studying halogenated bovine albumin in dogs, reported that the theoretical upper limit of iodine content for obtaining the slowest rate of disappearance of the protein is 0.2 percent, assuming one molecule of iodine incorporated per protein molecule.

Iodine-containing Compounds in Iodinated Casein

Several iodine-containing compounds are known to be present in iodinated casein. Ludwig and von Mutzenbecker (1939) isolated thyroxine, monoiodotyrosine and diiodotyrosine. In 1948 Pitt-Rivers reported the isolation of diiodohydroxybenzaldehyde. On the basis of chromatographic studies on iodinated casein hydrolysate, Hird and Trikojus (1948) identified the presence of diiodothyronine, and tentatively identified triiodothyronine by virtue of its position between diiodothyronine and thyroxine.

Metabolism studies on rats conducted by Gaddum (1929) indicate that diiodothyronine possesses 1/15 the biological activity of thyroxine. More recently, Gross and Pitt-Rivers (1952) have reported that synthetic triiodothyronine is 3 to

4 times as active as L-thyroxine. Their assay was conducted on thiouracil-treated rats.

EXPERIMENTAL

The Distribution of Iodinated Casein and a Comparison with Iodide in the Rat

The first in this series of experiments dealing with iodinated casein metabolism was designed to reveal the distribution and relative concentrations of injected iodinated casein in various tissues of the rat. Iodide concentrations, determined in a parallel experiment, were compared with the iodinated casein concentrations. It was shown by Wallace and Brodie (1937) that inorganic iodide occupies about the same volume in the organism as the extracellular water. According to Leblond (1951), inorganic iodide is concentrated in liver, stomach, and kidney in amounts which exceed the known capacity of the extracellular space. Any greater concentration of iodinated casein than iodide would therefore definitely establish the concentrating ability of the tissue.

Female albino rats which were purchased from Carworth Farms, New City, New York, were used in the experiment. Ten rats with an average weight of 168 grams were injected intravenously with 0.1 ml. solution of iodine-131 labelled iodinated casein (approx. 2 mg.). Nine rats with an average weight of 186 grams were injected by the same route with 0.1 ml. of

radioactive iodide.[1,2] Blood samples were taken 100 minutes after the injections, at which time the rats were sacrificed.

Preparation of Radioactive Iodinated Casein

The method described by Reineke et al (1943) was used with only minor modifications.

One half gm. of casein (purified, vitamin-free), 1 gm. of sodium bicarbonate (C.P.), and 18 ml. of distilled water were mixed in a 120 ml., capacity, heavy glass tube. Through a rubber stopper, fitted snugly into the mouth of the tube, a metal bushing was inserted to permit the passage of a brass stirring rod operated by an air-driven motor. The stirring rod was bent at the end to facilitate stirring.

In a separate tube radioactive iodine (I-131) solution was prepared from a radioactive iodide solution, by the addition of a small crystal of potassium iodate and one drop of concentrated phosphoric acid to about 1 ml. of radio iodide (I-131)*. A small iodide crystal was added and produced a color change, indicating that the iodine had been released from the iodide.

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[1] Purchased from Oak Ridge National Laboratory, Oak Ridge, Tenn.

[2] If the tissues are to be counted a day or two after the rats are sacrified, inject about 25 microcuries per rat.

* Ten to 30 millicuries of iodine-131 were used per preparation. This amount was high for the distribution experiment, but necessary for the metabolism experiment to be described.

The tube containing the casein was placed in a thermostatically controlled constant temperature water bath and the heating unit and stirring apparatus turned on, the latter being set for moderately rapid stirring. When the temperature of the bath had reached 40° C., stirring was stopped and the radioactive iodine solution, previously prepared, was added to the casein mixture. The tube which had contained the radio-iodine was rinsed with 1 ml. of distilled water and the rinsings added to the casein mixture. The total volume of water in the reaction mixture was about 20 ml. Stirring was resumed for several minutes after which time 0.086 gms. of iodine (I-127) crystals were added to the casein mixture. Stirring was again resumed and continued for 19 hours at a temperature of 70° C. The iodinated casein solution was then placed in a cellophane casing and dialyzed against running tap water for 5 days.

Injection and Tissue Sampling Procedure

The materials under investigation were administered intravenously into a branch of the femoral vein. The rats were anesthetized with ether and the vein exposed by cutting the skin on the inside of the upper part of a hind leg. The injection was usually made from a 1 ml. syringe with a three-quarter inch, 26 gauge hypodermic needle bent in a slight curve. Following the injection, the wound was closed with metal skin clips. One hundred minutes after the injection, the rats were again etherized and 1-3 ml. of blood

was withdrawn from the femoral vein, usually with a 5 ml. heparinized syringe and a half-inch 24 gauge hypodermic needle bent in a slight curve. The rats were then sacrificed with ether, wrapped in paper and stored under refrigeration. One portion of the blood was used to determine the hematocrit* and another portion was weighed wet in a tared 1.9 cm. (inside) diameter steel planchet, on a Gramatic balance. The blood was spread over the surface, by tilting the planchet, and dried at room temperature, usually overnight. Samples of other tissues taken from the rats included: gluteal muscle, lung, liver, spleen, kidney, stomach (cleaned), small intestine (empty or cleaned), and large intestine (empty or cleaned). Wet weight was determined on a torsion type balance or in a tared planchet on a Gramatic balance.** After weighing, the tissues were cut up on the planchet with a single edged razor blade and pinch forceps, and spread over the surface. Any tissue adhering to the cutting instruments was rubbed off onto the inside edge of the planchet. The tissue samples were then partially dried at room temperature, for several hours, and completely dried in an air oven at

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* Hematocrits were determined on the heparinized blood in Wintrobe hematocrit tubes which were centrifuged for 30 minutes at 2000 rpm.

** The wet weight of blood in the iodide experiment was computed from the dry weight. Tissue wet weights in the iodinated casein experiment were computed from the dry weight. The percent solid in each case was determined experimentally in rats from the same original group.

90-95° C. for about 30 hours. The dried samples were stored in a desiccator until they were reweighed to determine dry weight.

Radioactivity Measurements

The radioactivity in the samples was measured with a thin end-window Geiger-Müller tube in a lead shield and recorded by an electronic scaler. The radioactivity was computed as counts per second and corrected for physical decay of the isotope, self-absorption, and instrument change measured by counting a standard. Counts per gram of plasma were computed from the counts per gram of blood. The computations and corrections are shown in the appendix.

The data are presented in terms of

$$\frac{\text{counts per second per gram wet tissue}}{\text{counts per second per gram plasma}}$$

in order that comparisons might be made between the results of different experiments. The data are shown in table 1.

Results

Liver was found to concentrate the greatest amount of iodinated casein per gram of tissue, the ratio between liver and plasma being 5.65. The spleen also concentrated iodinated casein very effectively; its ratio was 3.45. The kidney had the next highest concentration with a ratio of 1.72. Of all the tissues studied, muscle showed the lowest concentration ratio of the iodinated casein, 0.18. The other tissues studied, with the ratios found, were: stomach 0.89, lung 0.66,

small intestine 0.54, and large intestine 0.36. A comparison of iodide concentration ratios shows the stomach to be highest with 0.50. The lowest concentration ratios were found in the muscle, 0.18 and cecal contents, 0.09. The other tissues studied, in order of iodide concentration ratio, were: spleen 0.44, liver 0.35, large intestine 0.35, kidney 0.33, small intestine 0.30, and lung 0.29. A graphic comparison (figure 1) of iodinated casein and iodide concentration ratios shows the iodinated casein to be more concentrated than iodide in all tissues studied, except muscle. In spite of the fact that casein is a large molecule (mol. wt. 188,000 Bayliss, 1943) which would not ordinarily be expected to cross cell membranes, these experiments show that it leaves the blood stream quite rapidly and is concentrated principally in the liver.

The Distribution of Slightly Iodinated Casein

From the results of the previous experiment it can be concluded that iodinated casein, prepared so as to have thyroidal activity, rapidly concentrates in various tissues in the rat; particularly liver, spleen, and kidney. It seemed of interest to find out whether or not this property could be attributed to the iodine content of the protein. The same procedure previously described for the study of iodinated casein distribution was carried out in this experiment with the following exceptions: no iodine-127 was added

Table 1. Concentration Ratios of Iodinated Casein and Iodide

Tissue	Iodinated Casein	Iodide
Gluteal muscle	0.18	0.18
Lung	0.66	0.29
Liver	5.65	0.35
Kidney	1.72	0.33
Spleen	3.45	0.44
Stomach	0.89	0.50
Small intestine	0.54	0.30
Large intestine	0.36	0.35
Cecal contents	----	0.09

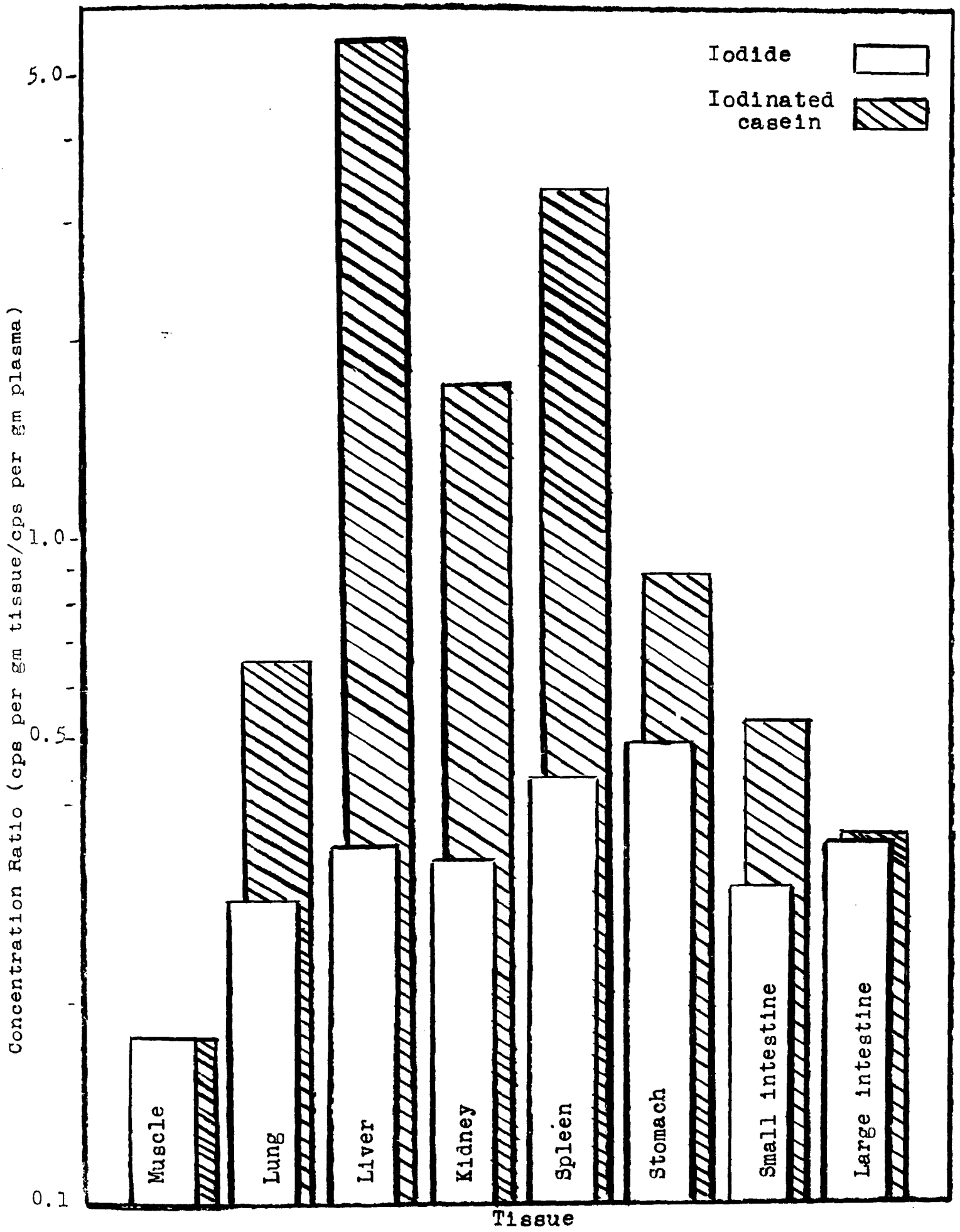

FIG. 1. CONCENTRATION RATIOS OF IODINATED CASEIN AND IODIDE

to the casein other than the iodine (about 0.0095 g.) in the small crystals of iodide and iodate, the radioiodine solution was neutralized with ammonium hydroxide to a pale yellow color before being added to the casein mixture, only 0.125 gm. sodium bicarbonate was mixed with the casein, and the average weight of the rats was 253 grams.

The principal difference between this experiment and the iodinated casein distribution study is the iodine content of the protein. The data are shown in table 2 and depicted grapnically in figure 2.

Results

The highest concentration ratios (2.56 and 2.36) were found in the spleen and stomach, respectively. The kidney also had a high concentration of 1.99. Muscle and cecal contents were lowest with concentration ratios of 0.23 and 0.35, respectively. In the other tissues studied, the order of concentration was: liver 1.15, large intestine 1.02, lung 0.71, and small intestine 0.62.

The most marked differences between the iodinated casein and the slightly iodinated casein distributions is the increased concentration in the stomach and large intestine, and the decreased concentration in the liver with the slightly iodinated casein.

Table 3. Concentration Ratios of Slightly Iodinated Casein

Tissue	Concentration Ratio
Gluteal muscle	0.23
Lung	0.71
Liver	1.15
Kidney	1.99
Spleen	3.55
Stomach	3.36
Small intestine	0.62
Large intestine	1.02
Cecal contents	0.35

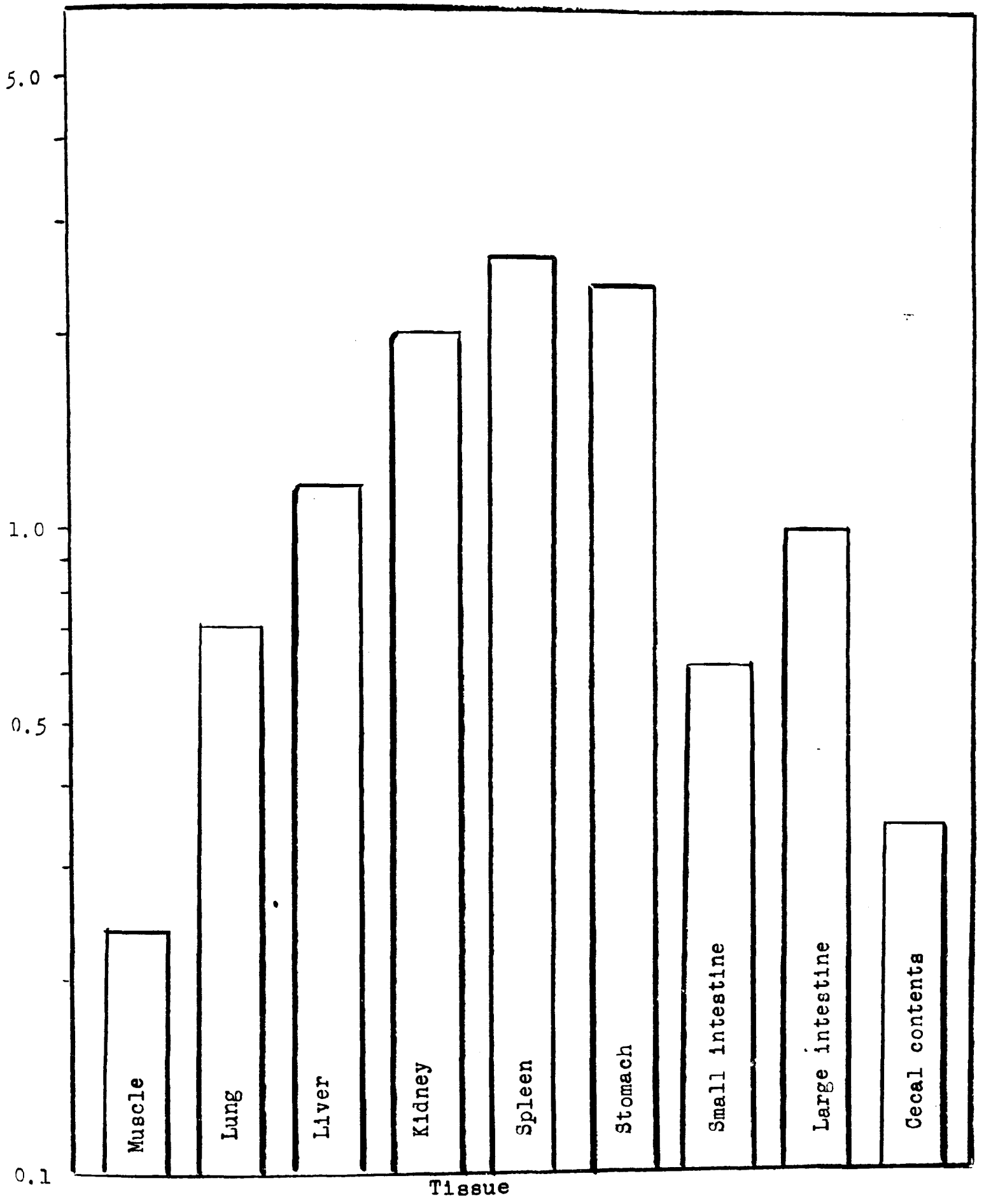

FIG. 2. CONCENTRATION RATIOS OF SLIGHTLY IODINATED CASEIN

The Metabolism of Iodinated Casein in the Rat

Intravenous Administration

The metabolic breakdown of injected iodinated casein in the rat was studied with a view toward characterizing the metabolites and determining their relative concentrations over a time series. If the iodinated casein was metabolized with the release of thyroxine-like iodine this could account for the thyroidal activity of iodinated casein. On the other hand, if the iodinated protein was metabolized without the release of thyroxine-like iodine the inference would be that thyroxine can exert its hormonal activity while combined in the protein.

Female albino rats which were purchased from Rockland Farms were used in the experiment. Four groups of two rats each, weighing about 230 grams per rat, were thyroidectomized and injected intravenously with 0.5 ml. of radioactive iodinated casein solution (approximately 10 mg.). The rats were thyroidectomized in order to prevent the formation of radio thyroxine by the thyroid gland, from iodine released as the iodinated casein was metabolized. Blood and liver samples were taken from each of two rats at four time periods: 1½, 12, 24, and 72 hours after injection. The samples were fractionated and computations carried out so that the fractions could be expressed as free thyroxine-like iodine, and protein combined thyroxine-like iodine.

Preparation of Radioactive Iodinated Casein

The procedure described in the first experiment was used with the following minor modifications: the radioiodine solution (approx. 15 mc.) was neutralized with ammonium hydroxide to a pale yellow color before being added to the casein mixture, and only 0.125 gm. of sodium bicarbonate was mixed with the casein.

Rat Thyroidectomy Procedure

The rat was partially anesthetized with 1 ml. of 0.6 percent sodium pentabarbital (nembutal), injected intraperitoneally. It was then fully anesthetized with ether and tied on its back to a wooden rat board. Four-tenths ml. of 10 percent calcium gluconate was injected subcutaneously, immediately before the operation, to prevent tetany, because the parathyroids are removed with the thyroid gland. An ether cone was used, as needed, during the course of the operation. The skin was cut parallel to and directly over the trachea and the longitudinal muscles and skin retracted on both sides with two hooks made from wire paper clips. Tension was maintained with rubber bands attached to the sides of the rat board. The exposed thyroid gland was teased from the trachea and blood vessels with a hypodermic needle and pinch forceps. Small wads of cotton were used to control bleeding. When the bleeding had stopped, the cotton was removed and the skin incision closed with metal skin clips.

The thyroparathyroidectomized rats were subsequently maintained on a standard diet (appendix X) with the addition of 1 percent calcium carbonate. They were given 0.4 ml. subcutaneous injections of 10 percent calcium gluconate two or three times each day for several days.

Injection and Tissue Sampling Procedures

The iodinated casein was injected into the femoral vein, usually before the rat came out from under the anesthesia administered during the thyroparathyroidectomy.

Collection of Blood and Liver Samples

At the appropriate time the rats were anesthetized with sodium pentabarbital and ether and tied to the rat board. Blood samples of about 5 ml. were withdrawn from the abdominal aorta, usually with a curved one and one-half inch 20 gauge hypodermic needle and a 10 ml. heparinized syringe. The blood was centrifuged for about 30 minutes at 2000 rpm to separate the plasma.

Following the withdrawal of the blood sample, the rat was sacrificed and a liver sample of a size estimated to weigh about 1 gm. was removed. This was placed in a tared 12 or 15 ml. pyrex centrifuge tube and weighed. Any storage necessary was under refrigeration.

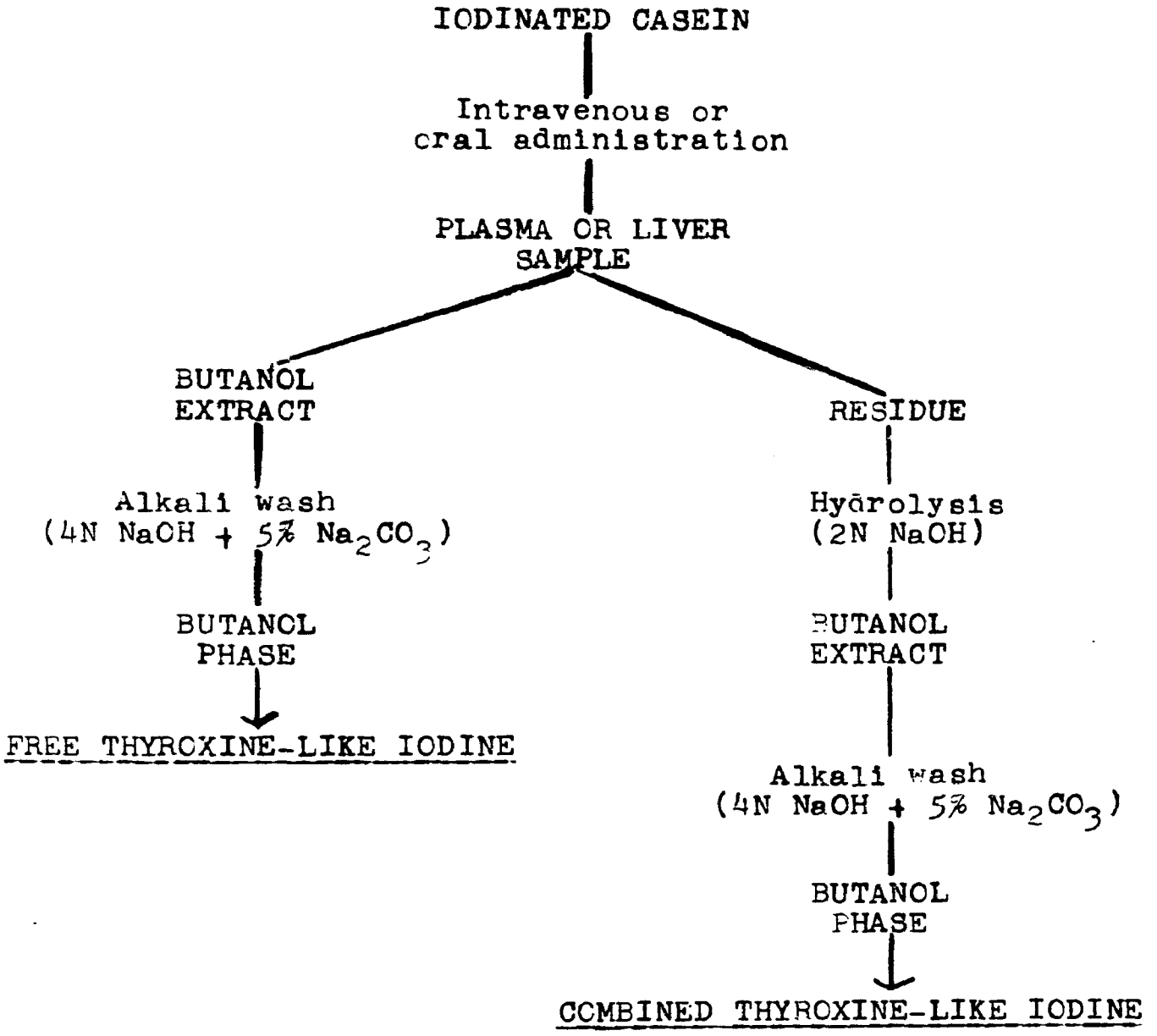

FIG. 3. FRACTIONATION OF PLASMA AND LIVER SAMPLES

Fractionation of Plasma and Liver Samples*

Extraction of unhydrolyzed plasma. One ml. of the plasma was transferred to another tube and extracted twice with 2 ml. of butanol each time. At each extraction the mixture was stirred for 2 minutes with a glass rod, stoppered and allowed to settle for 20 or more minutes at room temperature. The alcoholic extract was separated by centrifuging the mixture for 10 minutes at 2000 rpm. The entire extract was decanted into another tube and ½ ml. was transferred to a 2.5 cm., inside diameter, nickel plated planchet, dried at room temperature in a desiccator with the aid of a high vacuum pump, and a dry ice-acetone trap, and counted. The remaining extract was stored under refrigeration.

Extraction of unhydrolyzed liver. The liver was mashed in the tube with a glass rod, the process taking several minutes. Three ml. of butanol were added and mixed with the liver with the glass rod for 2 minutes. The tube was closed with a rubber stopper which had previously been soaked in butanol and wiped dry, and the suspension was shaken vigorously 24 times. The stopper was removed and the tube spun in the centrifuge for 10 minutes at 2000 rpm. The alcoholic extract was poured into another centrifuge tube and the liver residue extracted a second time with 2 ml. of butanol. Both extracts were combined, stoppered, and stored under refrigeration. One-half ml. of the combined extracts was transferred

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*A schematic representation is shown in figure 3.

to a planchet, dried as described earlier, and counted. The twice-extracted liver residue was dried in the centrifuge tube at room temperature as already described and saved for further treatment.

Hydrolysis of the Liver and Fractionation of the Hydrolysate

Five ml. of 2N sodium hydroxide were added to the dry liver residue in the centrifuge tube, an air-cooled reflux condenser was attached and the tube placed in a boiling water bath (approx. 99° C.) for 15 hours. The hydrolysate was cooled to room temperature and its pH adjusted to approximately 3.5 by the dropwise addition of 6N sulfuric acid; Brom Cresol Green was used as an internal indicator. The hydrolysate was then extracted twice with butanol, 3 ml. for each extraction, by mixing, shaking and centrifuging as already described. One-half ml. of the combined extracts was transferred to a planchet, dried as described earlier, and counted. The remainder of the combined extracts was stored under refrigeration.

Alkaline Washing of Butanol Extracts (Hydrolyzed and Unhydrolyzed)

Three ml. of unwashed butanol extract was transferred to another tube and extracted twice with 3 ml. volumes of a solution consisting of 4N sodium hydroxide and 5 percent sodium carbonate. Each extraction involved shaking 24 times, and centrifuging 10 minutes at 2000 rpm. The alkaline wash solution was removed with a tapered glass tube, attached to a

greased 5 ml. syringe with rubber tubing. One-half ml. of the twice-extracted butanol was transferred to a planchet, dried as described earlier, and counted.

Results

The data are presented in table 3 and depicted graphically in figures 4-7. The basic data are shown in appendix IV.

Combined thyroxine-like iodine in the plasma. As shown in figure 4, combined thyroxine-like iodine was rapidly cleared from the plasma; between the 1½ hr. and 24 hr. time intervals there was a 99 percent decrease, and by the 72-hr. interval only a very small amount of thyroxine-like iodine was found in the plasma.

Free thyroxine-like iodine in plasma. The free thyroxine-like iodine concentrations in the plasma (Figure 5) showed only a 21 percent decrease between the 1½ and 24 hour intervals. A large decrease, of 87 percent, occurred between 24 and 72 hours. The relatively constant concentration between 1½ and 24 hours and the large drop after 24 hours indicates that free thyroxine-like iodine was being released to the plasma from some other tissue or tissues and the amount supplied decreased and fell below the plasma turnover.

Percent free thyroxine-like iodine of the total thyroxine-like iodine, in the plasma. The percent free thyroxine-like iodine is computed by means of the following relationship:

$$\frac{\text{free thyroxine-like iodine}}{\text{free thyroxine-like iodine + combined thyroxine-like iodine}} \times 100$$

As shown in Figure 6, at the 1½ hour time interval a very small proportion of the total thyroxine-like iodine in the plasma was free. The percent free thyroxine-like iodine rose from 7 percent at 1½ hours to 75 percent at 12 hours. This high percent of free thyroxine-like iodine was maintained at the 24- and 72-hour intervals.

Combined thyroxine-like iodine in liver. There was only an 18 percent increase in the liver concentrations of combined thyroxine-like iodine between the 1½- and 24-hour intervals (Figure 7). On the other hand, there was a 91 percent decrease between 24 and 72 hours.

The sharp decline indicates that the rate at which combined thyroxine-like iodine had been supplied to the liver decreased. The supply of combined thyroxine-like iodine did not equal the turnover, and the liver concentrations could not be maintained at the level of the 1½- and 24-hour intervals.

Oral Administration

A recent report on the absorption characteristics of iodine-131 labelled iodinated casein (Campbell et al, 1950) indicates that a large amount of the iodine absorbed from the alimentary tract of rats and sheep is combined as iodinated casein or some butanol-insoluble metabolite; 10 hours after oral administration about 50% of the plasma radioactivity is butanol-insoluble.

Table 3. Metabolism of Iodinated Casein, Intravenous Administration

Time (hr.)	Rat No.	c.p.s. per ml. plasma: Free Thyroxine-like Iodine		c.p.s. per ml. plasma: Combined Thyroxine-like Iodine	
1½	1		20.9		407.6
	2		34.0		310.9
		av.	27.4	av.	359.2
24	5		30.5		3.68
	6		12.7		3.76
		av.	21.6	av.	3.72
		dec.*	21.1	dec.	98.9
72	7		2.64		0.80
	8		3.20		0.80
		av.	2.92	av.	0.80
		dec.	86.5	dec.	78.4
		c.p.s. per gm. liver			
1½	1		66.9		197.5
	2		58.1		231.0
		av.	62.5	av.	214.2
24	5	4	49.6		300.0
	6		38.9		206.7
		av.	44.2	av.	253.3
		dec.	29.2	incr.	18.2
72	7		12.9		25.2
	8		11.8		22.0
		av.	12.3	av.	23.6
		dec.	72.1	dec.	90.6

* Percent decrease from previous average count.

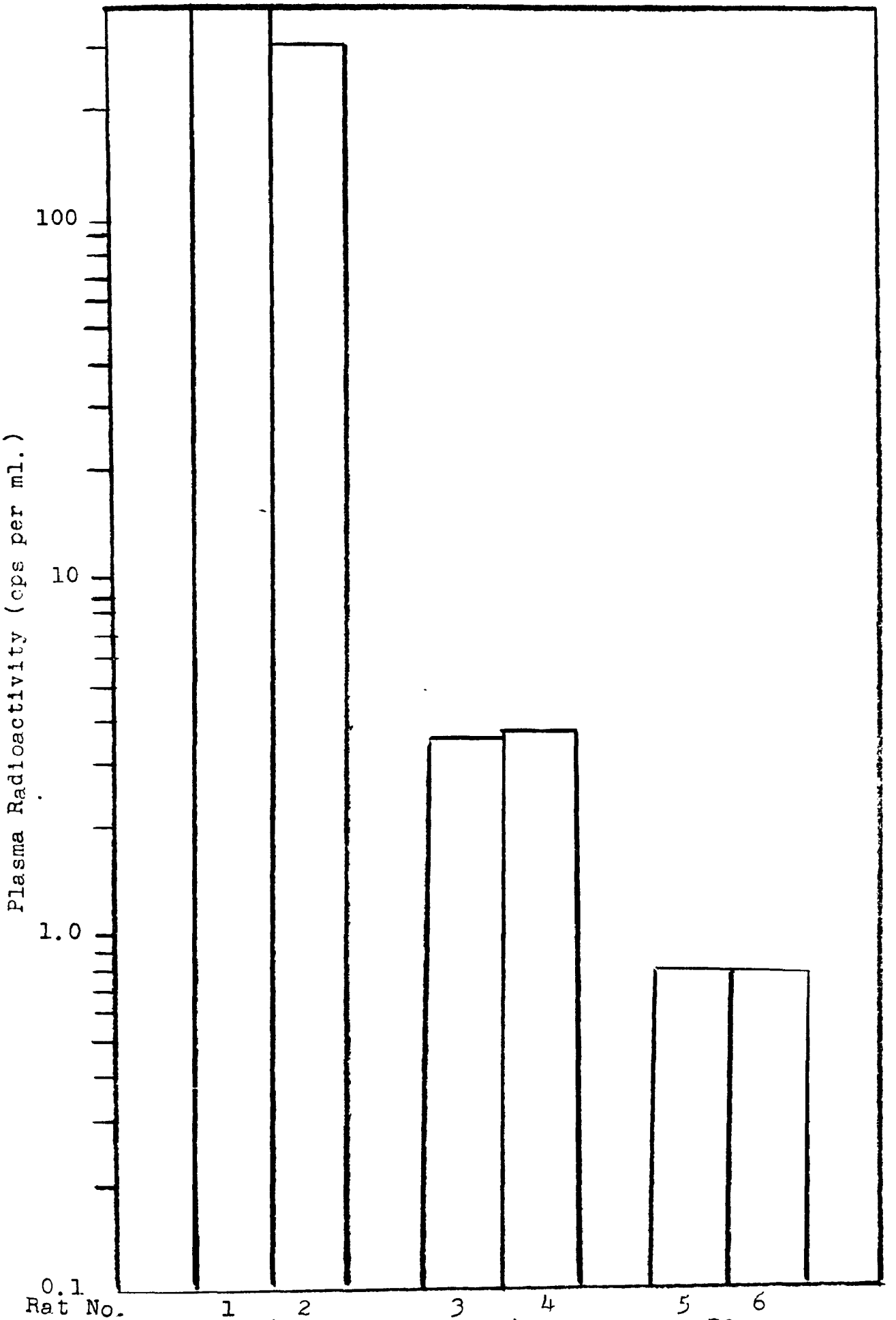

FIG. 4. COMBINED THYROXINE-LIKE IODINE IN PLASMA

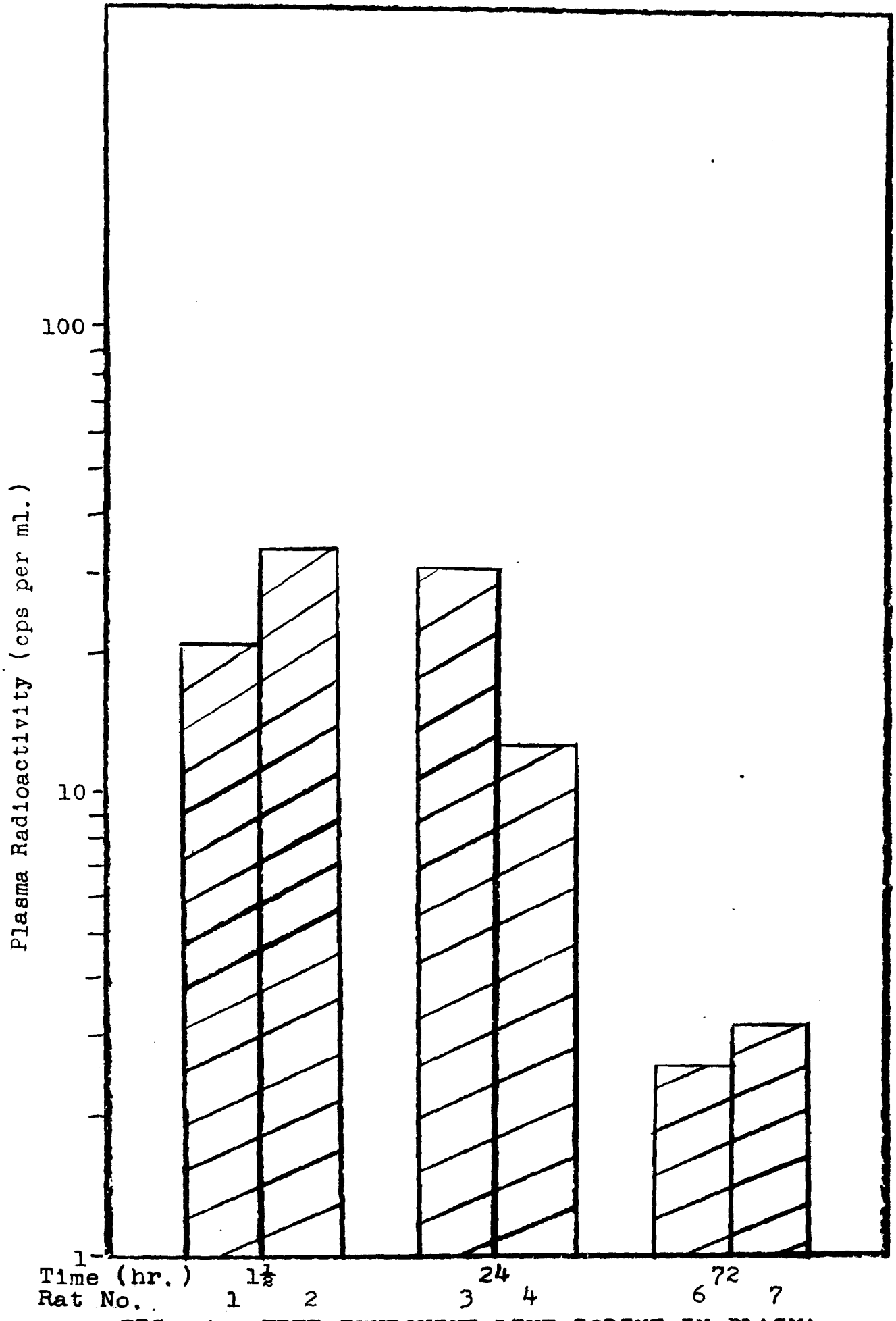

FIG. 5. FREE THYROXINE-LIKE IODINE IN PLASMA

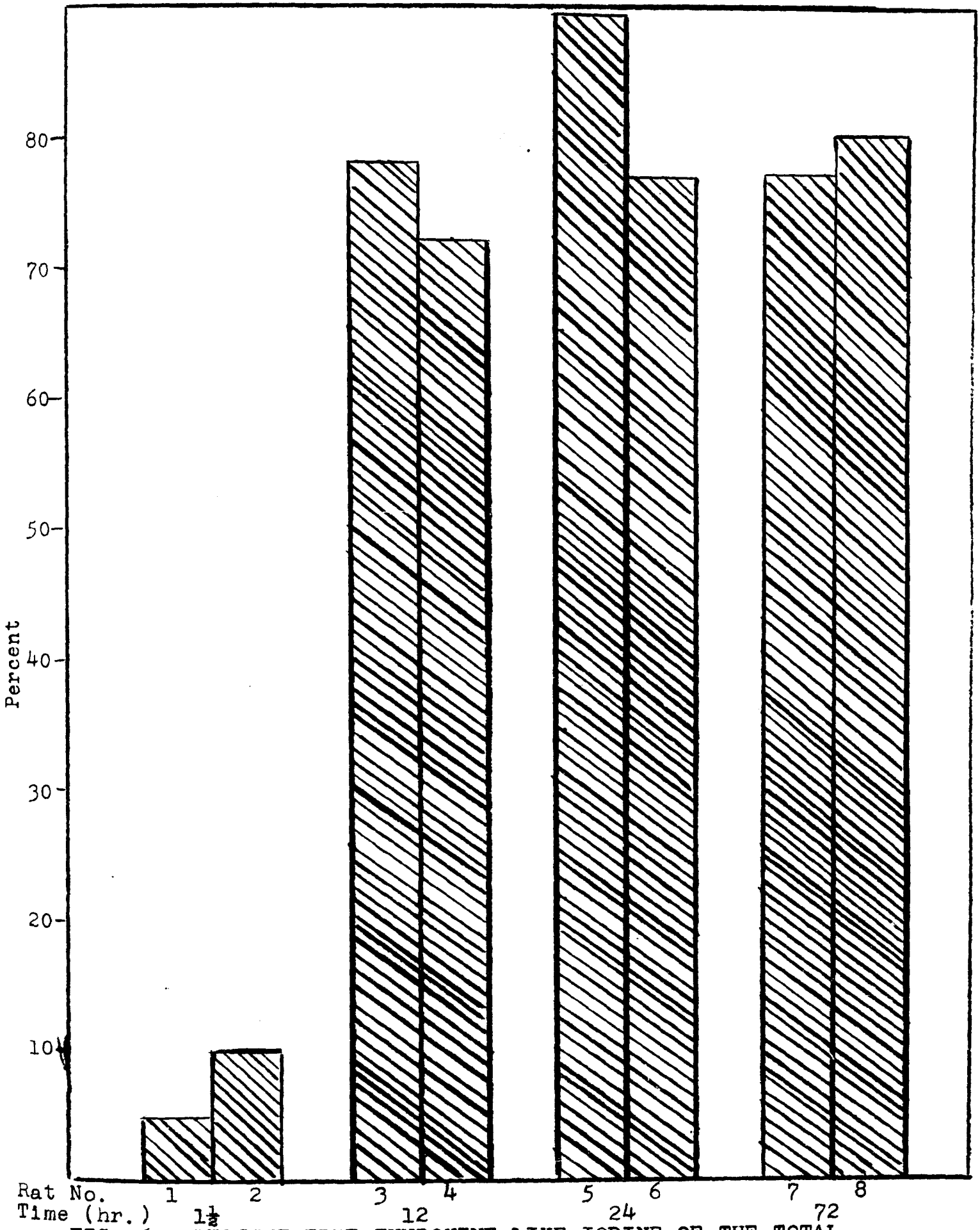

FIG. 6. PERCENT FREE THYROXINE-LIKE IODINE OF THE TOTAL THYROXINE-LIKE IODINE IN PLASMA

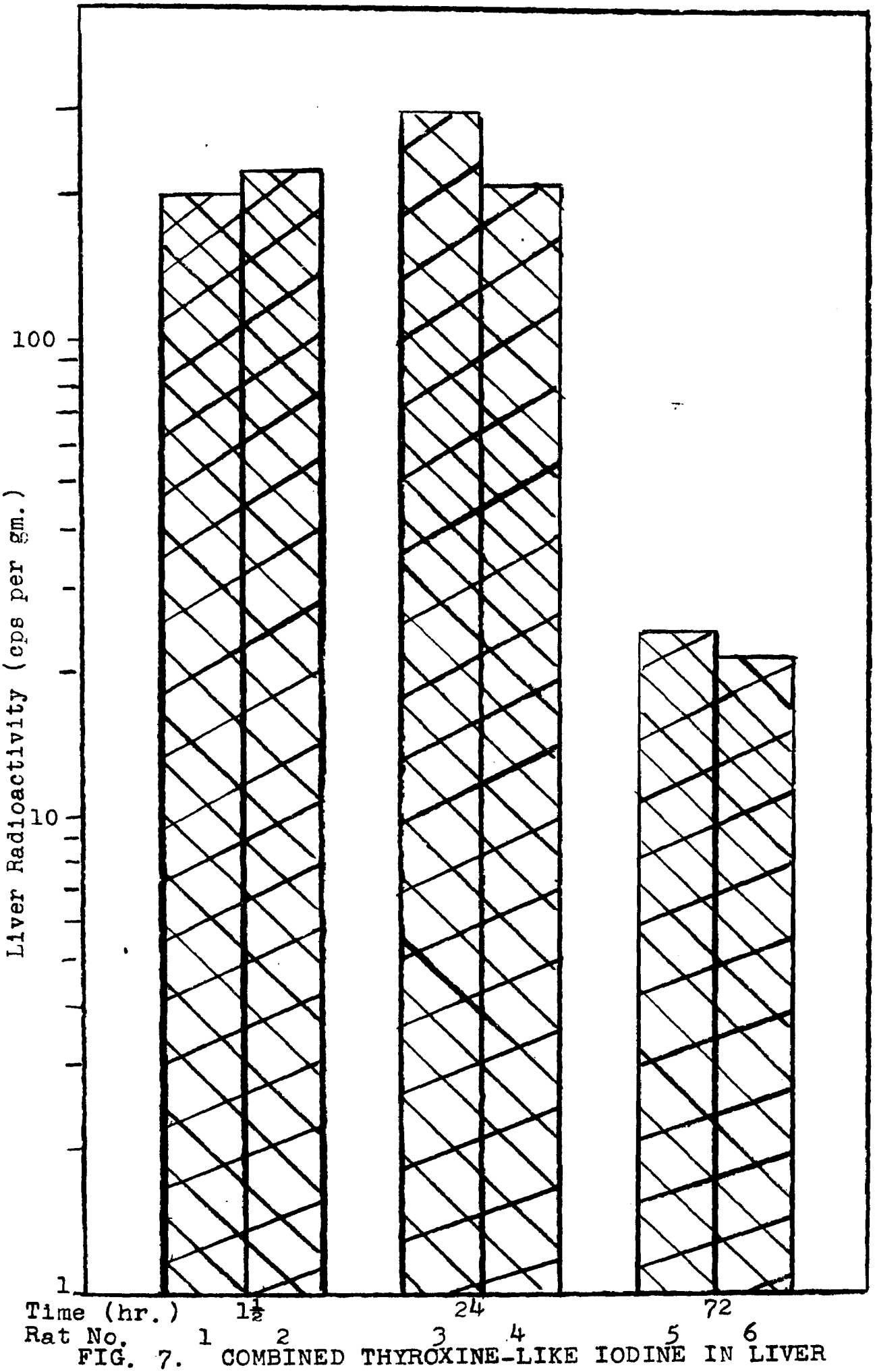

FIG. 7. COMBINED THYROXINE-LIKE IODINE IN LIVER

It seemed of interest to further characterize this fraction and determine the relationship between the time interval after oral administration of iodinated casein and the concentration of thyroxine-like iodine in the plasma.

The experiment was conducted on 3 groups of 2 male albino rats weighing about 337 grams each. The methods of the preceding experiment were used with the following exceptions: Two ml. volumes of iodinated casein were administered by stomach tube with the aid of a plastic tube connected to a hypodermic syringe, the blood samples were taken at 1½, 12 and 48 hours.

Results. Although the individual results of the experiment (table 4) were generally erratic, they suggest that the butanol-insoluble material entering the blood stream after oral administration of iodinated casein contains thyroxine-like iodine in combined form.

The percent free thyroxine-like iodine was computed by means of the following relationship,

$$\frac{\text{free thyroxine-like iodine}}{\text{free thyroxine-like iodine + combined thyroxine-like iodine}} \times 100$$

Figure 8 shows that more than 50 percent of the thyroxine-like iodine in the plasma, at the time intervals studied, was in the free, butanol-soluble, form.

Table 4. Metabolism of Iodinated Casein, Oral Administration

Time (hr.)	Rat No.	c.p.s. per ml. Plasma: Free Thyroxine-like Iodine	c.p.s. per ml. Plasma: Combined Thyroxine-like Iodine
1½	1	2.32	1.84
	2	14.4	5.52
12	3	24.1	3.60
	4	17.6	3.28
48	5	3.60	0.96
	6	2.08	1.12

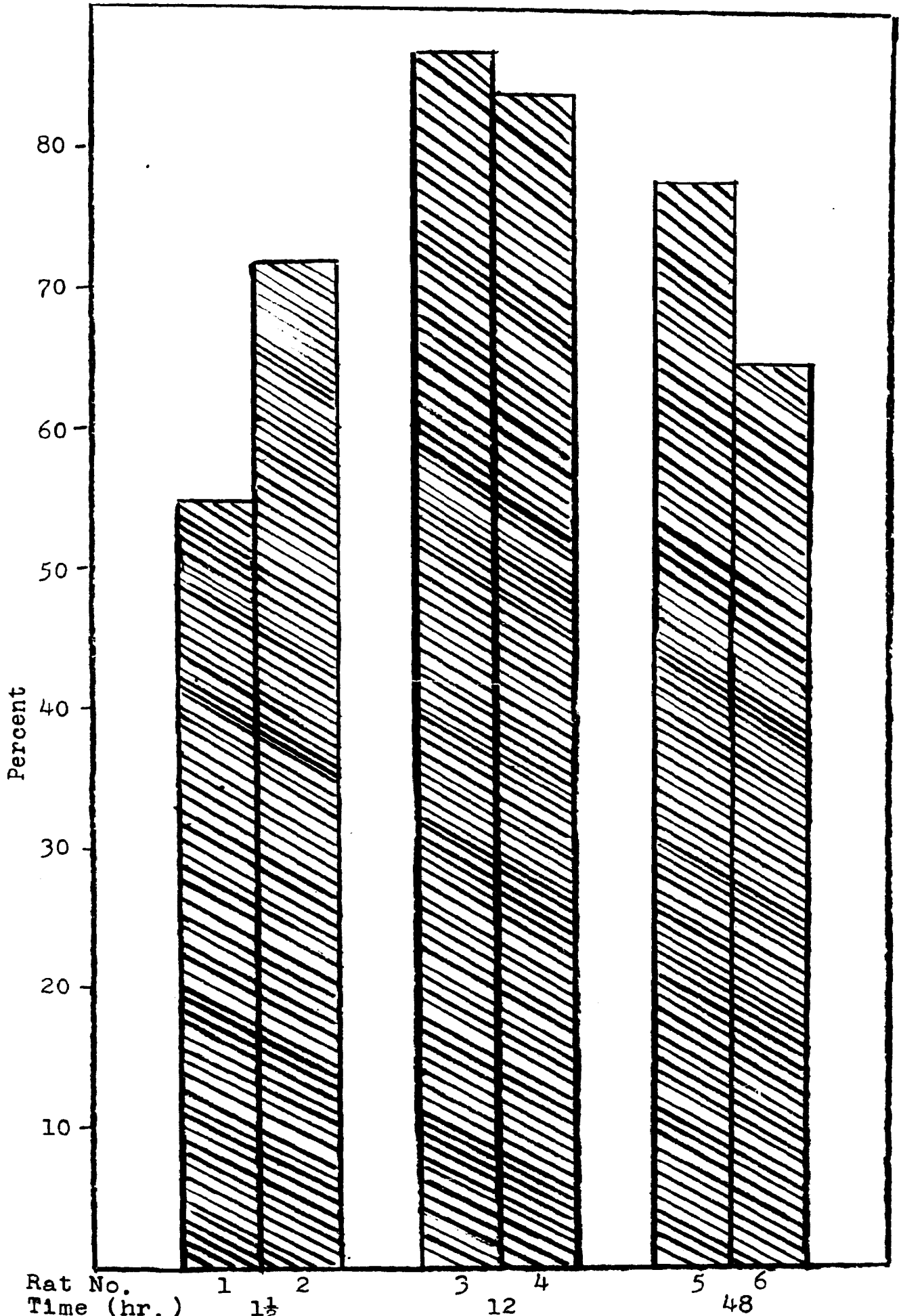

FIG. 8. PERCENT FREE THYROXINE-LIKE IODINE OF THE TOTAL THYROXINE-LIKE IODINE IN PLASMA

The Disappearance of Iodinated Casein from Plasma and the Appearance of its Metabolic Products in the Urine, in the Dog

The decreases in plasma and urine radioactivity in the dog, following the intravenous administration of radioactive iodinated casein, were compared in order to determine the influence of plasma iodinated casein concentration on urine radioactivity.

The relative molecular size of the radioactive material appearing in dog urine immediately following the injection of radioactive iodinated casein was determined by determining the urine radioactivity before and after dialysis.

Decrease in Plasma and Urine Radioactivity

A male dog was used to compare decreases in plasma and urine radioactivity. It was kept under anesthesia by the intravenous administration of 3 percent sodium pentobarbital, as needed.

Radioactive iodinated casein was prepared by the procedure described in the iodinated casein distribution experiment, except that the period of dialysis was 52 hours. One ml. (approx. 10 mg.) was administered into the femoral vein and blood samples of about 10 ml. were collected in heparinized test tubes from the cannulated carotid artery. The cannula was made from a 16 gauge hypodermic needle from which the hub had been removed with wire cutters. The straight

portion was extended several cm. by means of tight-fitting plastic tubing and plugged with a wire slightly longer than the distance between the mouth of the plastic extension and the point of the hypodermic needle.

At the appropriate time intervals the wire plug was removed and a blood sample taken. The plasma was separated by centrifugation, and radioactivity measurements were made on ml. portions with a shielded thin end-window Geiger-Muller tube and a scaler. The time periods studied and the percentages of the radioactivity at the first period are shown in table 5.

Urine samples were collected directly from the right ureter with the aid of a plastic catheter and radioactivity measurements of 1½ ml. portions were made with the apparatus just described. The data are shown in table 6 in terms of percentage of radioactivity at the first interval.

Results

Intravenously administered radioactive iodinated casein leaves the blood plasma at an extremely rapid rate. When compared with the blood plasma radioactivity at 1 minute, 83 percent was cleared from the plasma by the 17-minute interval. After the first large decrease, further clearance occurred at a much slower rate. Between the 17-minute and 362-minute intervals there was only a 6 percent further reduction in plasma radioactivity.

Table 5. Decrease in Blood Plasma Radioactivity

Time after admin. (min.)	c.p.s. per ml.	Percent of first sample
1	25.62	100
17	4.40	17.1
32	3.92	15.3
47	4.14	16.1
62	4.27	16.6
92	3.99	15.5
122	4.05	15.8
152	3.76	14.6
182	3.62	14.1
212	3.41	13.3
242	3.31	12.9
362	2.75	10.7

Table 6. Decrease in Urine Radioactivity

Collection period (min.)	Av. Time (min.)	c.p.s. per ml.	Percent of first sample
0-16	8	6.89	100
16-46	31	6.35	92.1
46-76	61	7.01	101
76-106	91	5.67	82.2
106-136	121	4.93	71.5
136-166	151	3.81	55.2
166-196	181	2.96	42.9
196-226	211	2.32	33.6
226-256	241	2.10	30.4
256-376	316	1.49	21.6
376-496	436	2.20	31.9

The decrease in urine radioactivity occurred more slowly; the radioactivity at 436 minute (av.) collection period was 32 percent of that at the 8-minute period.

The data suggest that iodinated casein was concentrated in the kidney during the rapid clearance from the blood plasma and excreted into the urine at a slower rate.

Radioactive Material in Urine

A 19-kilogram male dog was injected, intravenously (femoral vein), with 9 ml. of iodine-131 iodinated casein solution prepared by the method already described in the first experiment. Twenty-five minutes after the injection, the bladder of the unanesthetized dog was drained by means of a catheter attached to a hypodermic syringe. The urine was divided into two portions; one was placed in a cellophane casing and dialyzed against running tap water for 3 days, the other was stored under refrigeration. A ratemeter was then used to compare the radioactivity of 6 ml. of the dialyzed urine with 6 ml. of the undialyzed urine.

Results. The radioactivity was the same for both the dialyzed and the undialyzed urine, 900 counts per minute. It is therefore concluded that most or all of the radioactive material, which passes into the urine immediately following the injection of iodinated casein, is either the unaltered protein or a large undialyzable metabolite. This would eliminate free thyroxine and diiodotyrosine as possibilities.

DISCUSSION

The finding of Reineke et al (1942) that parentally administered iodinated casein exhibits hormonal activity led to the question of its mode of action; as stated in the beginning of this paper: Does the iodinated protein per se exert a biological effect or is it first metabolized with the release of thyroxine?

According to the results of the experiments reported herein, the thyroidal effect of iodinated casein is contingent on the metabolic splitting off of thyroxine. Direct evidence is not presented that iodinated protein per se is not hormonally effective. However, it is clear, from the results presented, that thyroxine-like iodine is hydrolyzed from iodinated casein in the liver and released to the plasma. That synthetic thyroprotein acts twice; once as the protein per se, and again as free thyroxine, would appear to be highly improbable.

Figure 1 shows the concentration of iodinated casein in various tissues of the rat 100 minutes after its intravenous administration. The highest concentration was found in the liver, where it was more than 10 times that of sodium iodide which had been administered similarly. Higher concentrations of iodinated casein than iodide were also found in

the spleen, kidney, lung, stomach, and small intestine. The concentration of iodinated casein in muscle and large intestine was similar to iodide. The high concentrations of iodinated casein in liver, kidney, and spleen is in accord with a similar report, by Haurowitz and Crampton (1952), on iodinated ovalbumin.

Liver concentrates a large proportion of injected iodinated casein due to its effective concentrating ability, and its large size, 11 gm. in a 200 gm. rat (Donaldson, 1924). In view of these considerations as well as the fact that liver is known to take part in protein metabolism it was assumed to be an important site of iodinated casein metabolism. Therefore, a comparison of the concentrations of iodinated casein and its thyroxine-like iodine metabolite in liver and plasma at different time intervals would indicate the rate of hydrolysis of iodinated casein to thyroxine, and permit estimations of relative turnover rates. The results show that the levels of iodinated protein and thyroxine in the liver and blood plasma are interdependent.

As long as the quantity of iodinated casein supplied to the liver equals the turnover, its concentration in the liver will be maintained at a constant level. Intravenously administered iodinated casein, represented by combined thyroxine-like iodine (Fig. 4), showed a 99 percent decrease in concentration in the plasma between the 1½- and 24-hour intervals. Since liver readily concentrates iodinated casein

(Fig. 1), it is presumed that a high rate of clearance from the blood plasma indicates a rapid uptake by the liver. The fact that the level of iodinated casein in the liver was maintained fairly constant between the 1½- and 24-hour intervals (Fig. 7) and decreased 91 percent by the 72-hour interval indicates that the rate at which the iodinated protein was supplied to the liver decreased below the turnover rate, and the liver concentration could not be maintained. That the supply had, in fact, decreased is shown in figure 3, where it can be seen that there was a 99 percent decrease in iodinated casein concentration in the plasma between the 1½- and 24-hour intervals. As a result of the decreased liver concentration of iodinated casein, the amount of thyroxine freed to the plasma decreased. This was followed by an 87 percent decrease in the plasma level of free thyroxine (Fig. 5).

A recent report by Campbell et al (1950) indicates that iodinated casein or some butanol-insoluble metabolite of it, as well as thyroxine, is absorbed into the blood plasma from the alimentary tract of rats and sheep. A further characterization of this butanol-insoluble fraction was carried out at 1½, 12, and 48 hours. The results indicated that this fraction contains thyroxine-like iodine in a combined form. It was also shown (Figure 8) that more than 50 percent of the thyroxine-like iodine in the plasma, at the time intervals studied, was in the free, butanol-soluble, form.

In a study of the distribution of slightly iodinated casein, it was found that this protein was concentrated in many of the same tissues that concentrate iodinated casein. Nevertheless, no tissue showed as effective a concentrating ability for slightly iodinated casein as that of liver for iodinated casein. Tissues with the high accumulations were: spleen, stomach, kidney, liver, large intestine, lung, and small intestine.

In figure 1 it was shown that the kidney accumulates iodinated casein rapidly. When these data are compared with the rapid rate of clearance of iodinated casein from the blood plasma of the dog (83 percent between the 1- and 17-minute intervals, table 5) and the slower rate of change in radioactivity in the urine (32 percent between the 8- and 436-minute collection periods, table 6), it is concluded that iodinated casein is rapidly accumulated in the kidney during its rapid clearance from the blood plasma and excreted into the urine at a slow rate. The rapid clearance of iodinated casein from the blood is in accord with the report of Campbell et al (1950) that 90 percent of the radioactivity of labelled iodinated casein is removed from the blood stream of sheep 7 hours after its intravenous administration.

The author's characterization of the radioactive material in the urine, 25 minutes after the intravenous injection of iodine-131 labelled iodinated casein, indicates that it is undialyzable.

The radioactivity in the urine may represent the intact iodinated protein or some large metabolite.

SUMMARY

1. Distribution studies of intravenously administered iodinated casein reveal that it is concentrated in the liver, spleen, kidney, lung, stomach, and small intestine. The highest concentration was found in the liver, where it was more than 10 times that of sodium iodide which had been administered similarly.
2. Slightly iodinated casein, administered intravenously, concentrated in the same tissues which were shown to concentrate iodinated casein. The slightly iodinated casein was not as concentrated in the liver but more concentrated in the stomach and large intestine than the iodinated casein.
3. When iodinated casein is administered intravenously, thyroxine-like iodine is hydrolyzed from the iodinated protein, in the liver, and released to the plasma. The amount of iodinated casein available to the liver and the plasma level of thyroxine-like iodine are interdependent.
4. Following the oral administration of iodinated casein, thyroxine-like iodine, in a combined form, can be found in the blood plasma. This may be the intact protein or some large metabolite.

5. Most of the thyroxine-like iodine appearing in the plasma, after the oral administration of iodinated casein, is in the free, butanol soluble, form.
6. Intravenously administered iodinated casein is rapidly accumulated in the kidney and there is a slow excretion of its metabolite.
7. The iodinated material excreted into the urine during the first 25 minutes is not dialyzable and may represent the intact iodinated protein or a large metabolite.

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Appendix I

THE DISTRIBUTION OF IODINATED CASEIN IN THE RAT

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm wet tissue	cps per gm tissue / cps per gm plasma
Blood	1	.4802	.1037	21.59	68.44	142.52	
	2	.4457	.0943	21.15	53.74	120.57	
	3	.3535	.0602	17.02	31.07	87.89	
	4	.5107	.1014	19.85	62.62	122.61	
	5	.4961	.1035	20.86	61.81	124.18	
	6	.5070	.1023	20.17	64.59	127.39	
	7	.4952	.0988	19.95	65.34	131.94	
	8	.3061	.0511	16.69	28.88	94.34	
	9	.2253	.0351	15.57	19.71	87.48	
	10	.4560	.0850	18.64	69.66	152.76	
			mean	19.14			
Plasma[2]	1	.2469			63.89	258.76	
	2	.2292			50.17	218.89	
	3	.1818			29.00	159.51	
	4	.2626			58.46	222.61	
	5	.2551			57.70	226.18	
	6	.2607			60.30	231.30	
	7	.2546			61.00	239.59	
	8	.1574			26.96	171.28	
	9	.1158			18.40	158.89	
	10	.2345			65.03	277.31	
Gluteal muscle[3]	1	.8600	.2120		50.48	58.69	.2268
	2	.5967	.1471		23.57	39.50	.1804
	3	.6263	.1544		23.16	36.97	.2317
	4	2.1342	.5261		90.54	42.42	.1905
	5	.4604	.1135		13.01	28.25	.1249
	6	.7813	.1926		20.16	25.80	.1115
	7	.4405	.1086		14.58	33.09	.1381
	8	.5857	.1444		24.31	41.50	.2422
	9	.4113	.1014		14.31	34.79	.2189
	10	.3979	.0981		15.00	37.69	.1359
						mean	.1800
						standard error[4]	.0154

THE DISTRIBUTION OF ICDINATED CASEIN IN THE RAT (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm. wet tissue	cps per gm tissue / cps per gm plasma
Lung	1	.5324	.1141		73.35	137.77	.5324
	2	.3345	.0717		53.01	158.47	.7239
	3	.4703	.1008		52.54	111.71	.7003
	4	.3658	.0784		41.04	112.19	.5039
	5	.4740	.1016		55.33	116.72	.5160
	6	.3826	.0820		50.88	132.98	.5749
	7	.3280	.0703		65.19	198.75	.8295
	8	.2827	.0606		35.49	125.53	.7328
	9	.5123	.1098		69.88	136.40	.8584
	10	.5025	.1077		92.78	184.63	.6657
						mean	.6637
						standard error	.0404
Liver	1	.8764	.2284		1050.56	1198.72	4.6325
	2	.7052	.1838		691.51	980.58	4.4797
	3	.6335	.1651		671.56	1060.07	6.6457
	4	.5652	.1473		639.69	1131.79	5.0841
	5	.7847	.2045		847.43	1079.94	4.7746
	6	.4735	.1234		462.92	977.65	4.2267
	7	.6354	.1656		826.42	1300.62	5.4285
	8	.8096	.2110		1059.33	1308.46	7.6393
	9	.7574	.1974		896.24	1183.31	7.4473
	10	.6795	.1771		1155.18	1700.04	6.1304
						mean	5.6488
						standard error	.3940
Kidney	1	.7061	.1514		---	---	---
	2	.6184	.1326		183.31	296.42	1.3541
	3	.6856	.1470		241.26	351.89	2.2060
	4	.6226	.1335		222.85	357.93	1.6078
	5	.6343	.1360		223.35	352.12	1.5568
	6	.6907	.1481		255.44	369.82	1.5988
	7	.6986	.1498		261.11	373.76	1.5599
	8	.5391	.1156		185.26	343.64	2.0063
	9	.6954	.1491		235.06	338.02	2.1273
	10	.5601	.1201		232.17	414.51	1.4947
						mean	1.7235
						standard error	.1949

THE DISTRIBUTION OF IODINATED CASEIN IN THE RAT (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm. wet tissue	cps per gm tissue / cps per gm plasma
Spleen	1	.5149	.1101		457.05	887.64	3.4303
	2	.3928	.0840		287.38	731.61	3.3423
	3	.5252	.1123		305.15	581.01	3.6424
	4	.4415	.0944		427.96	969.33	4.3543
	5	.4274	.0914		238.17	557.25	2.4637
	6	.4321	.0924		358.97	830.75	3.5916
	7	.4822	.1031		316.47	656.30	2.7392
	8	.3390	.0725		216.58	638.87	3.7299
	9	.5299	.1133		363.72	686.39	4.3199
	10	.3531	.0755		283.35	802.46	2.8937
						mean	3.4507
						standard error	.1974
Stomach	1	.2154	.0509		31.87	147.95	.5717
	2	.4705	.1112		49.34	104.86	.4790
	3	.2310	.0546		40.80	176.62	1.1072
	4	.1836	.0434		57.40	312.63	1.4043
	5	.2581	.0610		51.89	201.04	.8888
	6	.1967	.0465		47.71	242.55	1.0486
	7	.2496	.0590		41.24	165.22	.6895
	8	.3140	.0742		58.26	185.54	1.0832
	9	.3504	.0828		47.41	135.30	.8515
	10	.2691	.0636		59.02	219.32	.7908
						mean	.8914
						standard error	.0879
Small intestine	1	.5863	.1266		54.19	92.42	.3571
	2	.3478	.0751		45.01	129.41	.5912
	3	.3413	.0737		42.43	124.31	.7793
	4	.4400	.0950		47.43	107.79	.4842
	5	.5108	.1103		34.39	67.32	.2976
	6	.3876	.0837		53.35	137.64	.5950
	7	.2552	.0551		28.46	111.52	.4654
	8	.3223	.0696		42.22	130.99	.7647
	9	.4335	.0936		30.42	70.17	.4416
	10	.4409	.0952		71.17	161.41	.5820
						mean	.5358
						standard error	.0500

THE DISTRIBUTION OF IODINATED CASEIN IN THE RAT (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm. wet tissue	cps per gm tissue / cps per gm plasma
Large intestine	1	.2280	.0424		18.72	82.10	.3172
	2	.4975	.0925		35.49	71.33	.3258
	3	.3905	.0726		36.23	92.77	.5815
	4	.3399	.0632		28.86	84.90	.3813
	5	.4749	.0883		24.99	52.62	.2326
	6	.4486	.0834		25.14	56.04	.2422
	7	.5653	.1051		54.06	95.63	.3991
	8	.3668	.0682		28.18	76.82	.4485
	9	.2797	.0520		16.04	57.34	.3608
	10	.2711	.0504		22.30	82.25	.2965
						mean	.3585
						standard error	.0325

Rat No.	Body wt. (gm.)
1	170
2	166
3	180
4	166
5	174
6	182
7	158
8	160
9	184
10	151
mean	168

[1] Counts per second corrected for background, physical decay (appendix VI), and self-absorption (appendix VII).

[2] Plasma values were computed from the blood data.
gm. wet blood x 0.5143 = gm. wet plasma (appendix V)
blood cps x 0.9336 = plasma cps (appendix VIII).

[3] Wet wt. were computed from dry wt. Percent solid was determined in tissues in the iodide distribution experiment. Both experiments were run on rats from the same original group.

$$\frac{\text{Dry wt. (gm.)}}{\text{percent solid}} \times 100 = \text{wet wt. (gm.)}$$

[4] The standard error (S.E.) was computed from the standard deviation (S) as described in Dixon et al (1951) and Snedecor (1946), respectively.

$$\text{S.E.} = \frac{S}{\sqrt{N}} \qquad S = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}{N-1}}$$

Appendix II

THE DISTRIBUTION OF IODIDE IN THE RAT

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Pct. solid	cps[1]	cps per gm. wet tissue	cps per gm. tissue / cps per gm. plasma
Blood[2]	1	.1415	.0271		55.95	395.40	
	2	.2157	.0413		121.15	561.65	
	3	.1536	.0294		55.88	363.80	
	4	.1640	.0314		52.36	319.26	
	5	.2042	.0391		123.24	603.52	
	6	.2617	.0501		97.50	372.56	
	7	.1379	.0264		77.07	558.88	
	8	.1901	.0364		70.02	368.33	
	9	.1985	.0380		63.75	321.15	
Plasma[3]	1	.0727			34.64	476.47	
	2	.1109			75.02	676.46	
	3	.0789			34.60	438.52	
	4	.0843			32.42	384.57	
	5	.1050			76.32	726.85	
	6	.1345			60.38	448.92	
	7	.0709			47.72	673.06	
	8	.0977			43.36	443.80	
	9	.1020			39.48	387.05	
Gluteal muscle	1	.3585	.0945	26.35	33.13	92.41	.1939
	2	.574	.1370	23.86	55.22	96.20	.1422
	3	.4585	.1063	23.18	46.75	101.96	.2325
	4	.6935	.1678	24.19	65.66	94.67	.2461
	5	.5365	.1237	23.05	44.36	82.68	.1137
	6	.4640	.1494	32.19	31.99	68.94	.1535
	7	.7175	.1629	22.70	57.17	79.67	.1183
	8	.4475	.1021	22.81	36.09	80.64	.1817
	9	.6495	.1528	23.52	53.50	82.37	.2128
						mean	.1771
						standard error[4]	.0159
Lung	1	.3780	.0902	23.86	63.50	167.98	.3525
	2	.209	.0457	21.86	30.98	148.22	.2191
	3	.3000	.0664	22.13	56.99	189.96	.4331
	4	.4160	.0910	21.87	57.32	137.78	.3582
	5	.3260	.0678	20.79	48.65	149.23	.2053
	6	.2300	.0510	22.17	22.16	96.34	.2146
	7	.5005	.1037	20.71	63.52	126.91	.1885
	8	.3570	.0782	21.90	54.15	151.68	.3417
	9	.6550	.1156	17.64	67.62	103.23	.2667
			mean	21.43		mean	.2866
						standard error	.0288

THE DISTRIBUTION OF IODIDE IN THE RAT (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Pct. solid	cps[1]	cps per gm. wet tissue	cps per gm. tissue / cps per gm. plasma
Liver	1	.4920	.1256	25.52	98.56	200.32	.4204
	2	.7645	.1980	25.89	118.51	155.01	.2291
	3	.6410	.1708	26.64	118.32	184.58	.4209
	4	.8710	.2262	25.97	117.29	134.66	.3501
	5	.5560	.1392	25.03	94.04	169.13	.2326
	6	.8195	.2193	26.76	131.06	159.92	.3562
	7	.8710	.2339	26.85	145.07	166.55	.2474
	8	.7320	.1960	26.77	173.20	236.61	.5331
	9	.7615	.1915	25.14	108.93	143.04	.3695
			mean	26.06		mean	.3510
						standard error	.0338
Kidney	1	.565	.1140	20.17	103.70	183.53	.3851
	2	.5560	.1180	21.22	84.62	152.19	.2249
	3	.5045	.1077	21.34	88.06	174.54	.3980
	4	.8025	.1686	21.00	138.76	172.90	.4495
	5	.6420	.1391	21.66	106.80	166.35	.2288
	6	.6545	.1401	21.40	93.40	142.70	.3178
	7	.7730	.1658	21.44	103.00	133.24	.1979
	8	.5740	.1208	22.78	88.57	154.30	.3476
	9	.6520	.1433	21.97	97.66	149.78	.3869
			mean	21.44		mean	.3262
						standard error	.0298
Spleen	1	.443	.0880	19.86	90.57	204.44	.4290
	2	.4220	.0794	18.81	58.98	139.76	.2066
	3	.484	.0907	18.73	81.97	169.35	.3861
	4	.5525	.1332	24.10	187.34	339.07	.8833
	5	.4955	.1115	22.50	82.58	166.65	.2292
	6	.3570	.0733	20.53	54.50	152.66	.3400
	7	.6105	.1293	21.17	105.94	173.52	.2578
	8	.4285	.1005	23.45	107.53	250.94	.5654
	9	.4185	.0977	23.34	105.51	252.11	.6513
			mean	21.38		mean	.4387
						standard error	.0746
Stomach	1	.143	.0323	22.58	28.11	196.57	.4125
	2	.2500	.0479	19.16	40.23	160.92	.2378
	3	.3390	.0792	23.36	61.06	180.11	.4107
	4	.4935	.1240	25.12	215.14	435.94	1.1335
	5	.6185	.1432	23.15	138.96	224.67	.3091
	6	.3720	.0901	24.22	60.01	161.31	.3593
	7	.3620	.0895	24.72	57.78	159.61	.2371
	8	.3010	.0708	23.52	100.22	332.95	.7502
	9	.6410	.1723	26.87	157.96	246.42	.6366
			mean	23.63		mean	.4985
						standard error	.0980

THE DISTRIBUTION OF IODIDE IN THE RAT (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Pct. solid	cps[1]	cps per gm. wet tissue	cps per gm. tissue / cps per gm. plasma
Small intestine	1	.0775	.0150	19.35	11.35	146.45	.3073
	2	.2140	.0452	21.12	28.29	132.19	.1954
	3	.170	.0282	16.58	20.32	119.52	.2725
	4	.2780	.0616	22.15	41.38	148.84	.3870
	5	.4355	.0837	19.21	72.24	165.87	.2282
	6	.0900	.0226	25.11	10.59	117.66	.2620
	7	.0975	.0267	27.38	12.42	127.38	.1892
	8	.1415	.0273	19.29	27.88	197.03	.4439
	9	.3760	.0910	24.20	55.00	146.27	.3779
			mean	21.59		mean	.2959
						standard error	.0300
Large intestine	1	.2590	.0503	19.42	45.07	174.01	.3652
	2	.1280	.0238	18.59	18.29	142.89	.2112
	3	.342	.0599	17.51	52.89	154.64	.3526
	4	.2060	.0459	22.28	54.71	265.58	.6905
	5	.2835	.0621	21.90	48.90	172.48	.2372
	6	.1685	.0319	18.93	24.03	142.61	.3176
	7	.1390	.0276	19.85	14.58	104.89	.1558
	8	.2840	.0509	17.92	49.94	200.96	.4528
	9	.2840	.0596	20.98	39.71	139.82	.3612
			mean	18.59		mean	.3493
						standard error	.0523
Cecal contents	1	.6665	.1512	22.68	85.59	55.02	.1154
	2	.6675	.1395	20.89	71.06	45.51	.0672
	3	.7057	.1526	21.62	85.10	53.28	.1214
	4	.6727	.1340	19.91	76.50	49.04	.1275
	5	.7293	.1645	22.55	79.27	48.38	.0665
	6	.6879	.1509	21.93	72.42	45.77	.1019
	7	.7130	.1429	20.04	73.96	45.88	.0681
	8	.7352	.1390	18.90	61.77	37.86	.0853
	9	.5998	.1054	17.57	54.05	36.01	.0929
			mean	20.67		mean	.0940
						standard error	.0074

	Body wt. (gm.)	Hematocrit
1	180	.50
2	188	.46
3	184	.47
4	202	.50
5	168	.42
6	202	.50
7	196	.40
8	176	.49
9	184	.49
mean	186	mean .47

THE DISTRIBUTION OF IODIDE IN THE RAT (cont.)

[1] Counts per second corrected for background, physical decay (appendix VI), and self-absorption (appendix VII).

[2] Wet wt. were computed from dry wt. Percent solid (19.14) was determined in tissues in the iodinated casein distribution experiment. Both experiments were run on rats of the same original group.

$$\frac{\text{Dry wt. (gm.)}}{\text{percent solid}} \times 100 = \text{wet wt. (gm.)}$$

[3] Plasma values were computed from the blood data.
gm. wet blood x 0.5143 = gm. wet plasma (appendix V)
blood cps x 0.6193 = plasma cps (appendix IX)

[4] See footnote 4, appendix I.

Appendix III

THE DISTRIBUTION OF SLIGHTLY IODINATED CASEIN IN THE RAT

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm. wet tissue	cps per gm tissue / cps per gm plasma
Plasma	1	.2661	.0264	9.92	17.88	67.19	
	2	.1592	.0144	9.04	12.13	76.19	
	3	.1212	.0131	10.80	7.59	62.62	
	4	.1592	.0162	10.17	25.40	159.54	
	5	.1781	.0204	11.45	15.73	88.32	
	6	.1808	.0147	8.13	9.65	53.37	
	7	.1330	.0132	9.92	12.04	90.52	
	8	.1811	.0163	9.00	7.88	43.51	
	9	.1114	.0100	8.97	8.85	79.44	
	10	.1282	.0125	9.75	11.89	92.74	
			mean	9.71			
Gluteal muscle	1	.7522	.1902	25.28	13.90	18.47	.2748
	2	.7402	.1811	24.46	15.13	20.44	.2682
	3	.6734	.1639	24.33	9.06	13.45	.2147
	4	.8438	.2098	24.86	18.00	21.33	.1336
	5	.7315	.1891	25.85	7.76	10.60	.1200
	6	.7716	.2017	26.14	13.05	16.91	.3168
	7	.6908	.1632	23.62	13.15	19.03	.2102
	8	.8335	.2055	24.65	10.99	13.18	.3029
	9	.8402	.2118	25.20	18.19	21.64	.2724
	10	.8765	.2274	25.94	19.01	21.68	.2337
			mean	25.03			mean .2347
							standard error[2] .0209
Lung	1	.5379	.1233	22.92	26.97	50.13	.7460
	2	.4638	.1112	23.97	26.56	57.26	.7515
	3	.5889	.1357	23.04	29.87	50.72	.8099
	4	.6518	.1458	22.36	61.64	94.56	.5927
	5	.4417	.1039	23.52	24.93	56.44	.6390
	6	.4582	.1023	22.32	19.66	42.90	.8038
	7	.4648	.0995	21.40	28.29	60.86	.6723
	8	.4880	.1121	22.97	16.66	34.13	.7844
	9	.3778	.0766	20.27	18.50	48.96	.6163
	10	.3825	.0797	20.83	23.54	61.54	.6635
			mean	22.36			mean .7079
							standard error .0254

THE DISTRIBUTION OF SLIGHTLY IODINATED CASEIN (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm wet tissue	cps per gm tissue / cps per gm plasma
Liver	1	.7726	.1991	25.77	49.05	63.48	.9447
	2	1.0130	.2478	24.46	75.99	75.01	.9845
	3	.9566	.2579	26.96	72.09	75.36	1.2034
	4	.9392	.2528	26.91	135.78	144.56	.9061
	5	1.0371	.2692	25.95	179.70	173.27	1.9618
	6	.7939	.2149	27.06	43.14	54.33	1.0142
	7	1.0136	.2835	27.96	94.84	93.56	1.0335
	8	.9233	.2733	29.60	45.08	48.82	1.1220
	9	1.1029	.2813	25.50	87.47	79.30	.9982
	10	.8556	.2234	26.11	108.90	127.27	1.3723
			mean	26.62		mean	1.1540
						standard error	.0997
Kidney	1	1.0303	.2274	22.07	131.14	127.28	1.8742
	2	.8185	.1662	20.30	118.66	144.97	1.9027
	3	.8968	.1961	21.86	158.71	176.97	2.8260
	4	.6333	.1432	22.61	94.94	149.91	.9396
	5	.8538	.1902	22.27	137.53	161.07	1.8237
	6	.8400	.1907	22.70	147.15	175.17	3.2821
	7	.8568	.1952	22.78	148.59	173.42	1.9158
	8	.9793	.2366	24.16	81.54	83.26	1.9135
	9	.7769	.1703	21.92	89.96	115.79	1.4575
	10	.7352	.1607	21.85	131.34	178.64	1.9262
			mean	22.25		mean	1.9861
						standard error	.2060
Spleen	1	.3856	.0900	23.34	70.84	183.71	2.7341
	2	.4449	.0987	22.18	111.31	250.19	3.2837
	3	.3700	.0877	23.70	77.44	209.29	3.3422
	4	.3248	.0732	22.53	50.27	154.77	.9701
	5	.2877	.0667	23.18	67.50	234.61	2.6563
	6	.4751	.1092	22.98	92.72	195.15	3.6565
	7	.3461	.0760	21.95	86.87	250.90	2.7727
	8	.4026	.0995	24.71	33.01	81.99	1.8843
	9	.3670	.0806	21.96	58.66	159.83	2.0119
	10	.4327	.0942	21.77	89.40	206.60	2.2277
			mean	22.83		mean	2.5539
						standard error	.2542

THE DISTRIBUTION OF SLIGHTLY IODINATED CASEIN (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm wet tissue	cps per gm tissue / cps per gm plasma
Stomach	1	.2693	.0731	27.14	55.03	204.34	3.0412
	2	.3429	.0880	25.66	84.51	246.45	3.2346
	3	.2267	.0726	32.02	40.52	178.73	2.8541
	4	.2786	.0697	25.01	35.45	127.24	.7975
	5	.3154	.0813	25.77	36.11	114.48	1.2961
	6	.3189	.0845	26.49	62.12	194.79	3.6498
	7	.3222	.0947	30.45	58.70	182.18	2.0125
	8	.3631	.1106	24.65	41.60	114.56	2.6329
	9	.4461	.1100	27.93	78.07	175.00	2.2029
	10	.1815	.0507	23.53	31.18	171.79	1.8523
			mean	26.86		mean	2.3573
						standard error	.2826
Small intestine	1	.1606	.0378	23.53	11.18	69.61	1.0360
	2	.2868	.0653	22.76	12.44	43.37	.5692
	3	.1989	.0441	22.17	7.60	38.21	.6101
	4	.1367	.0298	21.79	4.52	33.06	.2072
	5	.3162	.0740	23.40	21.15	66.88	.7572
	6	.2653	.0582	21.93	14.32	53.97	1.0112
	7	.2366	.0490	20.71	8.60	36.34	.4014
	8	.5052	.1085	21.47	18.09	35.80	.8227
	9	.3782	.0841	22.23	13.08	34.58	.4325
	10	.3543	.0768	21.67	11.04	31.16	.3359
			mean	22.16		mean	.6183
						standard error	.0895
Large intestine	1	.3048	.0861	28.24	16.14	52.95	.7880
	2	.2519	.0644	25.56	15.78	62.64	.8221
	3	.2961	.0859	29.01	15.92	53.76	.8585
	4	.1614	.0397	24.59	4.69	29.05	1.8208
	5	.2119	.0557	26.28	22.09	104.24	1.1802
	6	.2612	.0589	22.54	19.81	75.84	1.4210
	7	.3165	.0688	21.73	24.20	76.46	.8446
	8	.2885	.0709	24.57	14.61	50.64	1.1638
	9	.2294	.0539	23.49	12.83	55.92	.7039
	10	.2592	.0531	20.48	15.20	58.64	.6323
			mean	24.64		mean	1.0235
						standard error	.1177

THE DISTRIBUTION OF SLIGHTLY IODINATED CASEIN (cont.)

Tissue	Rat No.	Wet wt. (gm.)	Dry wt. (gm.)	Percent solid	cps[1]	cps per gm wet tissue	cps per gm tissue / cps per gm plasma
Cecal contents	1	.8640	.1587	18.36	24.28	28.10	.4182
	2	.8011	.1374	17.15	21.70	27.08	.3554
	3	.7473	.1291	17.27	22.27	29.80	.4758
	4	.8598	.1686	19.60	25.32	29.44	.1845
	5	.8059	.1494	18.53	18.80	23.32	.2640
	6	.8074	.1508	18.67	19.94	24.69	.4626
	7	.9326	.1680	18.01	31.24	33.49	.3699
	8	1.0045	.1743	17.35	17.04	16.96	.3897
	9	.8860	.1861	21.00	23.02	25.98	.3270
	10	.7503	.1362	18.15	17.70	23.59	.2543
			mean	18.40		mean	.3501
						standard error	.0296

Rat No.	Body wt. (gm.)
1	268
2	244
3	258
4	226
5	239
6	259
7	240
8	336
9	235
10	228
mean	253

[1] Counts per second corrected for background, physical decay (appendix VI), and self-absorption (appendix VII).

[2] See footnote 4, appendix I.

Appendix IV

THE METABOLISM OF IODINATED CASEIN, INTRAVENOUS ADMINISTRATION

Time (hr.)[1]		1½				12			
Rat No.		1	1	2	2	3	3	4	4
Tissue		Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma
wt. or vol.		1.11 gm	1 ml	0.92 gm	1 ml	1.56 gm	1 ml	1.48 gm	1 ml
cps per ½ ml. butanol[2]									
	A[3]	27.59	11.20	22.29	13.37	11.97	5.75	13.78	11.30
	B	7.43	2.62	5.35	4.26	5.15	2.22	4.84	2.86
	HA	125.72	224.33	108.55	172.12	31.33	4.08	41.62	8.97
	HB	18.28	50.95	17.72	38.87	5.73	0.62	7.40	1.13
cps per gm or ml of tissue									
	A	248.5	89.6	242.2	106.9	76.7	46.0	93.1	90.4
	B	66.9	20.9	58.1	34.0	33.0	17.7	32.7	22.8
	HA	1359.0	1794.6	1415.8	1376.9	240.9	32.6	337.4	71.7
	HB	197.5	107.6	231.0	310.9	44.0	4.96	57.0	9.04

THE METABOLISM OF IODINATED CASEIN, INTRAVENOUS ADMINISTRATION (cont.)

Time (hr.)[1]		24				72			
Rat No.		5	5	6	6	7	7	8	8
Tissue		Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma
Wt. or vol.		1.82 gm	1 ml	1.69 gm	1 ml	1.12 gm	1 ml.	1.59 gm	1 ml
cps per ½ml. butanol[2]									
	A[3]	32.32	12.90	23.40	8.00	3.73	0.61	4.43	0.61
	B	9.04	3.82	6.59	1.59	1.45	0.33	1.89	0.40
	HA	202.33	7.73	129.79	5.74	20.87	1.48	30.95	1.76
	HB	45.51	0.46	29.12	0.47	2.36	0.10	2.92	0.10
cps per gm or ml of tissue									
	A	177.5	103.2	138.4	64.0	33.3	4.88	27.8	4.88
	B	49.6	30.5	38.9	12.7	12.9	2.64	11.8	3.20
	HA	1334.0	61.8	921.5	45.9	223.5	11.8	233.5	14.0
	HB	300.0	3.68	206.7	3.76	25.2	0.80	22.0	0.80

[1] Rats 3 and 4 were injected with a different preparation of iodinated casein than had been administered to the other rats in this experiment. The preparation also had a different radioactivity.

[2] Counts per second corrected for background and physical decay (Appendix VI).

[3] A unwashed butanol extract
B alkaline washed butanol extract
HA unwashed butanol extract of hydrolysate
HB alkaline washed butanol extract of hydrolysate

Appendix IV (cont.)

THE METABOLISM OF IODINATED CASEIN, ORAL ADMINISTRATION

Time (hr.)		1½				12			
Rat No.		1	1	2	2	3	3	4	4
Tissue		Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma
Wt. or vol.		2.21 gm	1 ml	1.98 gm	1 ml	1.13 gm	1 ml	1.50 gm	1 ml
cps per ½ ml butanol[1]									
	A[2]	4.01	6.60	16.55	37.75	6.90	19.07	5.03	16.61
	B	0.11	0.29	0.77	1.80	1.12	3.02	0.70	2.21
	HA	2.09	5.48	9.40	23.48	4.56	9.61	3.46	7.80
	HB	0.19	0.23	0.48	0.69	1.87	0.45	0.17	0.41
cps per gm or ml of tissue									
	A	18.1	52.8	83.5	302.0	61.0	152.5	3.35	132.8
	B	0.49	2.32	3.88	14.4	9.91	24.1	4.66	17.6
	HA	11.3	43.8	56.9	187.8	48.4	76.8	27.6	62.4
	HB	1.03	1.84	2.90	5.52	19.8	3.60	1.36	3.28

THE METABOLISM OF IODINATED CASEIN, ORAL ADMINISTRATION (cont.)

Time (hr.)		48			
Rat No.		5	5	6	6
Tissue		Liver	Plasma	Liver	Plasma
Wt. or ml.		2.00 gm	1 ml	2.01 gm	1 ml
cps per ½ ml butanol[1]					
	A[2]	1.59	1.83	2.47	3.74
	B	0.30	0.45	0.07	0.26
	HA	1.15	2.26	1.07	2.74
	HB	0.12	0.12	0.05	0.14
cps per gm or ml of tissue					
	A	7.95	14.6	12.2	29.9
	B	1.50	3.60	0.34	2.08
	HA	6.90	18.0	6.36	21.9
	HB	0.72	0.96	0.29	1.12

[1] Counts per second corrected for background and physical decay (appendix VI).

[2] A unwashed butanol extract
B alkaline washed butanol extract
HA unwashed butanol extract of hydrolysate
HB alkaline washed butanol extract of hydrolysate

Appendix V

Standard Rat Blood Values and Computation of Correction Factors

Values

Specific gravity of blood (females)[1]	1.054
Specific gravity of plasma (mixed sex)[1]	1.023
Percent solid in blood, by wt.[2]	19.14
Percent plasma in blood, by vol.[3]	53.

Computations

Iodinated Casein Distribution Experiment

1 gm. wet blood/1.054 = 0.9487 ml. blood
0.9487 ml. blood x 0.53 = 0.5028 ml. plasma
0.5028 ml. plasma x 1.023 = 0.5143 gm. wet plasma
therefore, gm. wet blood x 0.5143 = gm. wet plasma

Iodide Distribution Experiment

1 gm. dry blood/0.1914 = 5.224 gm. wet blood
5.224 gm. wet blood/1.054 = 4.956 ml. blood
4.956 ml. blood x 0.53 = 2.626 ml. plasma
2.626 ml. plasma x 1.023 = 2.686 gm. wet plasma
therefore, gm. dry blood x 2.686 = gm. wet plasma

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[1] Albritton, 1952
[2] Iodinated casein distribution experiment, this paper.
[3] Iodide distribution experiment, this paper.
1.00 - hematocrit

Appendix VI

CORRECTION FOR PHYSICAL DECAY

$$\frac{A}{A_o} = \text{antilog } \frac{(-0.693)(t)}{(T)(2.3)}$$

A	present activity
A_o	original activity
t	time elapsed since A
T	half-life of isotope (I-131, 192 hr.)

For the purpose of correcting for physical decay, the 24-hour day was divided into 4-hour periods, except in the case of The Metabolism of Iodinated Casein, Intravenous Administration, where 8-hour periods were used.

Appendix VII

Correction for Self-absorption

Due to the varying thicknesses of the tissue samples on which radioactivity determinations were made, it was deemed necessary to correct the observed cps for self-absorption due to tissue.

Iodine-131 iodinated casein was administered intravenously to a large rat. One hundred minutes after the injection the rat was sacrificed and a portion of the liver was removed. This was finely divided and mixed well with the aid of a single edge razor blade and pinch forceps. Varying amounts of the mixture were evenly distributed on tared 1.9 cm. diameter steel planchets. The liver on the planchets varied from amounts barely covering the surface to completely filling the planchet. The tissue samples were dried for 14 hours at 90° C. in an air oven, cooled to room temperature in a desiccator, and weighed on a Gramatic balance. Radioactivity determinations were made by the same method described in the iodinated casein and iodide distribution experiment. The planchet was placed in the same position in the shield (middle shelf) so that the geometry was reproduced.

The log of the radioactivity per tissue weight (cps per mg.) was found to vary as the tissue weight (mg.) From the basic data radioactivity per mg. was computed for samples between 0 and 550 mg. from the regression equation

$$\log \hat{Y} = \log \bar{Y} + \frac{\Sigma xy}{\Sigma x^2} (X - \bar{X})$$

correction factors were then computed from the equation,

$$\text{correction factor} = \frac{\hat{Y} \text{ at zero mg.}}{\hat{Y} \text{ at x mg.}}$$

The product of the appropriate correction factor and the experimentally determined radioactivity of a sample equals the radioactivity corrected for self-absorption.

Appendix VII (cont.)

SELF-ABSORPTION CORRECTION FACTORS FOR IODINE IN TISSUE

	Tissue dry wt. (mg.)	cps*	cps per mg.		Deviations from mean		Square of deviations	Product of deviations
	X		Y	log Y	y	x	x^2	xy
	19.3	22.83	1.18	.0718	.1056	-54.8	3003.04	-5.78
	32.1	35.21	1.09	.0374	.0712	-42.0	1764.00	-2.99
	42.9	45.40	1.05	.0211	.0549	-31.2	973.44	-1.71
	81.4	72.46	.890	-.0507	-.0169	7.3	53.29	-0.12
	102.7	82.70	.805	-.0942	-.0604	28.6	817.96	-1.72
	101.1	85.00	.840	-.0758	-.0420	27.0	729.00	-1.13
	139.5	99.68	.714	-.1463	-.1125	65.4	4277.16	-7.35
Sum	519.0			-.2367			11617.89	-20.80
Mean	74.14			-.0338				

$$\log \hat{Y} = \log \bar{Y} + \frac{\Sigma xy}{\Sigma x^2} (X - \bar{X})$$

$$\log \hat{Y} = -.0338 + \frac{-20.80}{11617.89} (X - 74.14)$$

$$\hat{Y} = \text{antilog} (.0989 - .00179 X)$$

* Counts per second corrected for background and physical decay (appendix VI)

SELF-ABSORPTION CORRECTION FACTORS FOR IODINE IN TISSUE (cont.)

Tissue wt. (gm)*	cps per mg.	Correction factor	Tissue wt. (gm)	cps per mg.	Correction factor
.000	1.25	1.00	.280	.396	3.15
.010	1.20	1.04	.290	.380	3.28
.020	1.15	1.08	.300	.364	3.43
.030	1.11	1.12	.310	.350	3.57
.040	1.06	1.17	.320	.335	3.73
.050	1.02	1.22	.330	.322	3.88
.060	.980	1.27	.340	.309	4.04
.070	.941	1.32	.350	.296	4.22
.080	.903	1.38	.360	.284	4.40
.090	.866	1.44	.370	.273	4.57
.100	.830	1.50	.380	.262	4.77
.110	.798	1.56	.390	.251	4.98
.120	.765	1.63	.400	.241	5.18
.130	.734	1.70	.410	.231	5.41
.140	.705	1.77	.420	.222	5.63
.150	.676	1.84	.430	.213	5.86
.160	.649	1.92	.440	.204	6.12
.170	.623	2.00	.450	.196	6.37
.180	.598	2.09	.460	.188	6.64
.190	.550	2.18	.470	.181	6.90
.200	.528	2.27	.480	.173	7.22
.210	.507	2.36	.490	.166	7.53
.220	.486	2.46	.500	.160	7.81
.230	.467	2.57	.510	.153	8.16
.240	.448	2.67	.520	.147	8.50
.250	.430	2.79	.530	.141	8.86
.260	.412	2.90	.540	.135	9.25
.270	.396	3.03	.550	.130	9.61

*The tissue weight is shown in gm., instead of mg., for convenience only.

Appendix VIII

The Distribution of Radioactivity in Blood 100 Minutes After the Intravenous Administration of Iodine-131 Labelled Iodinated Casein

The distribution of radioactivity between the plasma and red cell fractions of blood was determined 100 minutes after the intravenous administration of iodine-131 labelled iodinated casein. This information was used to compute the error involved when all the radioactivity is assumed to be in the plasma.

The iodinated casein was prepared by the same method described in the iodinated casein distribution experiment, with the modifications outlined in the iodinated casein metabolism experiment. The administration was by way of the femoral vein, and the blood sample (3.4 ml.) was removed from the abdominal aorta. One ml. of blood was pipetted onto a nickel planchet and the remainder centrifuged to determine the hematocrit and to separate the plasma. One ml. of the plasma was pipetted onto a nickel planchet and both blood and plasma samples were dried at room temperature. Radioactivity measurements were made with a gamma tube in conjunction with a ratemeter.

The results indicate that 93.36 percent of the radioactivity per gram of blood is in the plasma fraction. Therefore the radioactivity per gram of blood multiplied by 0.9336 equals the radioactivity in the plasma fraction. The data and computations follow:

Data

Sample	Counts per minute[1]
plasma	3470
blood	1970
percent plasma[2]	53

Computations

$$\frac{(\text{cpm per ml. plasma})(\text{ml. plasma per gm. wet blood})}{(\text{cpm per ml. blood})(\text{ml. blood per gm. wet blood})} \times 100 =$$

percent of cpm per gm. of wet blood, in the plasma

cpm per ml. plasma	3470
cpm per ml. blood	1970
ml. plasma per gm. wet blood[3]	.5028
ml. blood per gm. wet blood[3]	.9487
percent of cpm per gm. of wet blood, in the plasma	93.36

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[1] corrected for background

[2] (1.00 - hematocrit) x 100

[3] appendix V

Appendix IX

The Distribution of Radioiodide Between Plasma and Red Cells

The distribution of radioactivity between the plasma and red cell fractions of blood was determined 100 minutes after the intravenous administration of radioiodide (I-131). This information was used to compute the error involved when all the radioactivity is assumed to be in the plasma.

The experiment was conducted on 3 large rats. Ether was used as the anesthetic and injections were made into the femoral vein. Blood samples were taken from the abdominal aorta with a heparinized syringe, transferred to 12 to 15 ml. centrifuge tubes and centrifuged for 30 minutes at 2000 rpm. The plasma was separated from the red cells and samples of each were taken and weighed wet in tared planchets on a gramatic balance. The samples were dried in an air oven at 90-95° C. and weighed dry.

Computations

cps per gm. red cells, wet wt.	0.65
cps per gm. plasma, wet wt.	1.00
gm. plasma per gm. blood, wet wt.*	0.514
gm. red cells per gm. blood, wet wt.**	0.486

0.65 x 0.486 = 0.3159 cps in the red cells of 1 gm. of the wet blood

1.00 x 0.514 = 0.5140 cps in the plasma of 1 gm. of the wet blood

therefore, 61.9 percent of the cps per gm. of wet blood is in the plasma

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* Appendix V.
** 1.000 - 0.514

THE DISTRIBUTION OF RADIOIODIDE BETWEEN PLASMA AND RED CELLS(cont.)

Tissue	Rat No.	Wet Wt.	Dry Wt.	Percent Solid	cps*	cps per gm. wet tissue	cps per gm red cells / cps per gm plasma
Plasma	1	.3168	.0265	8.36	45.02	142.10	
		.1414	.0126	8.91	21.60	152.75	
		.1259	.0113	8.97	19.53	155.12	
					mean	149.99	
	2	.2378	.0203	8.53	59.01	248.14	
		.1993	.0177	8.88	51.48	258.30	
					mean	253.22	
	3	.3331	.0270	8.10	86.07	258.39	
		.2950	.0242	8.20	71.98	244.00	
					mean	251.19	
Red cells	1	.3784	.1276	33.72	36.95	97.64	.650
	2	.3710	.1312	35.36	54.70	147.43	
		.3925	.1371	34.92	61.27	156.10	
					mean	151.76	.598
	3	.3810	.1281	33.62	69.05	181.23	
		.2906	.0938	32.27	49.17	169.20	
					mean	175.21	.697
						mean	.648

Rat no.	Rat wt. (gm.)
1	435
2	232
3	245

* counts per second corrected for background, physical decay (Appendix VI) and self absorption (Appendix VII).

Appendix X

4 Kilo "Hoppert" Stock Ration

Yellow corn meal (Thoman)	1400 gm.
Ground whole wheat (Thoman)	1000 gm.
Whole milk powder (Borden)	800 gm.
Linseed oil meal (Thoman)	400 gm.
Alfalfa leaf meal (Thoman)	240 gm.
Brewer's yeast (Strain G) (A. Busch)	120 gm.
Table salt (iodized)	40 gm.