Reprinted from ENDOCRINOLOGY
Vol. 79, No. 3, September, 1966
pp. 511-523

Alterations in Thyroid Physiology During Pneumococcal Septicemia in the Rat[1]

GEORGE E. SHAMBAUGH, III,[2] AND WILLIAM R. BEISEL[3]
U. S. Army Medical Unit, Fort Detrick, Maryland

ABSTRACT. The impact of acute infection on thyroid physiology was investigated in rats infected with a type-specific pneumococcus. Infected rats showed a lower thyroidal ^{131}I uptake than healthy controls at all time periods studied. Unlike in the rat, thyroidal ^{131}I uptake curves in the infected and healthy mouse and guinea pig were superimposable. Thyroidal ^{131}I release in the infected rat was significantly delayed when compared to similar release curves in uninfected animals. Although the thyroid of the infected rat responded to a physiologic dose of exogenous TSH, both its uptake and release of ^{131}I showed an absolute reduction. Serum TSH levels measured by bio-assay were not altered during infection. The disappearance time of ^{131}I-T_4 from blood of infected rats was significantly shorter than that of healthy controls, while disappearance times of blood ^{131}I-T_3 were unchanged. Organ radioactivity was measured following serial sacrifice after the administration of ^{131}I-T_4 or ^{131}I-T_3 to healthy and infected rats. In the T_4 group radioactivity in livers from infected rats was consistently lower than in healthy animals. In both the infected T_3 and T_4 groups, thyroidal iodine concentration was decreased. Kidney radioactivity in the T_3 and T_4 groups was unchanged by infection. Although infection altered bowel motility, with a resultant delay in the appearance of radioactivity in the colon of rats given ^{131}I-T_3 or ^{131}I-T_4, total body disappearance of the labeled hormones, followed over a period of several days, was not appreciably altered. The decrease in thyroidal function during infection appeared to be related to an intrinsic defect within the gland itself. This concept was supported by a marked fall in the PBI and circulating unbound thyroxine. The decrease in serum ^{131}I-T_4 $t_{\frac{1}{2}}$, an increase in the per cent of unbound thyroxine, and an increase in T_3 resin uptake were postulated to be the result of a decrease in T_4 binding by serum proteins during infection. The failure of serum TSH levels to change appeared to be related to a decreased pituitary response to alterations in circulating unbound thyroxine. In spite of these changes, no specific alteration in thyroid physiology could be demonstrated that would differentiate infection from other nonspecific stresses in the rat. (*Endocrinology* **79**: 511, 1966)

THE RESPONSE of the thyroid gland in generalized infection has been the subject of only a few reports compared to the volume of literature on intrinsic diseases of the thyroid. Although diminished basal metabolic rates were reported as early as 1926 in children and adults following pneumonococcal pneumonia (1), no basic research into the mechanisms of thyroid alterations in generalized infection appeared until 1955, when Sternberg demonstrated a depression of thyroidal ^{131}I uptake in the mouse infected with *Coccidioides immitis* (2). This report was followed by a comprehensive paper by Reichlin and Glaser (3), who infected rats by an intratracheal injection of streptococci and found the thyroidal release of ^{131}I to be delayed. They postulated that reduced TSH output, associated with lowered food intake during infection, might contribute to the reduction of thyroidal activity in a manner similar to the depression of TSH which occurred in starved rats (4). Badrick and Brimblecombe noted an electric shock-induced decrease of ^{131}I uptake in the thyroid of the hypophysectomized rat, which appeared to result from direct suppression of the gland (5). Following exposure to cold, heat, fasting, formalin injection, anoxia, nephrectomy, intestinal injury and avitaminosis, rats have responded with a decrease of thyroi-

Received November 15, 1965.

[1] In conducting the experiments described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

[2] Major, M.C.

[3] Lt. Colonel, M.C.

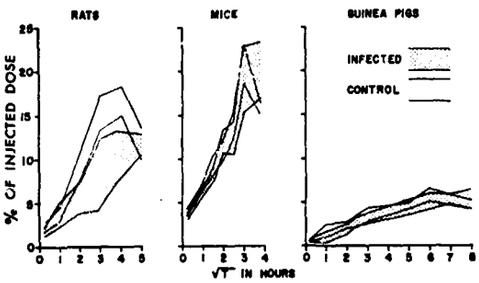

FIG. 1. Thyroidal ^{131}I uptake during pneumococcal septicemia in rats, mice and guinea pigs. The data are plotted as the mean ±95% confidence limits (C.L.) of control and infected groups. Ten rats, 20 mice, or 10 guinea pigs in each group were studied at each time period (see text).

dal ^{131}I uptake (6–9). A decrease in serum PBI has also been reported in rats subjected to various stresses (8–10).

We have attempted to define the interrelationships of pituitary-thyroid gland and peripheral thyroid hormone responses during experimentally induced infection to determine if changes were similar to those reported in other stresses.

Herein are presented data derived from an extensive series of individual studies, each designed to explore the impact of acute infection on a specific aspect of thyroid physiology. The rat was used as the experimental animal, and a virulent strain of pneumococcus was used as the infecting agent in all experiments. To evaluate species differences, certain comparative studies were also conducted with the mouse and guinea pig.

Materials and Methods

Male Dunning Fisher rats weighing from 130 to 300 g were used. All mouse experiments employed male albino Swiss animals weighing 25–35 g. Rats and mice were fed Purina rat chow, stated by the manufacturer to contain 1 mg/kg of iodine, and were given tap water *ad lib.* For the McKenzie TSH assay, mice were given Ken-L Biskit (0.75 mg/kg iodine) for 6 weeks before use. Hartley strain male guinea pigs weighing 360–550 g were fed a Rockland Guinea Pig Diet (Teklad, Inc.) containing 1.9 mg/kg iodine. All animals were housed in a controlled temperature room (22 ± 1 C) and were exposed to artificial light 10 hr a day.

The infecting agent was an encapsulated *Diplococcus pneumoniae* type I-A strain 5, which was stored in brain-heart infusion broth at −50 C. Virulence of this encapsulated organism was maintained by mouse passage. Twenty-two hr before use, the stock culture was thawed and 0.5 ml was transferred to 4.5 ml brain-heart infusion broth to which had been added 0.5 ml rabbit serum and sheep erythrocytes. After incubation for 18 hr, 0.5 ml of this culture was transferred to 4.5 ml of fresh medium and incubated for 4 hr, following which 1.0 ml of the culture diluted in 9.0 ml of tryptose phosphate broth was serially diluted. Rats and mice were infected subcutaneously in the back with 1.0 and 0.5 ml, respectively, of the 10^{-8} dilution, which averaged 5–15 organisms/ml by plate counting; controls were injected with a similar volume of sterile media.

Responses of the rat and mouse to infection were similar although onset was more rapid in the mouse. In the rat bacteremia developed by 24 hr and rectal temperatures became elevated to 101–103 F. The height of fever did not correlate well with either the clinical status or the length of survival after infection, which averaged 60 hr. At autopsy the most impressive finding was an extensive subcutaneous edema about the neck, back and feet; pneumonia was not evident. Approximately 5% of infected rats recovered; data from these animals were excluded from the study results. Guinea pigs were more resistant to infection with *D. pneumoniae:* a dose of 1.8×10^8 organisms/ml was required to produce a similar illness.

Iodide-^{131}I, 1.28 μc/ml (Oriodide), was used for all uptake and release experiments. Labeled triiodothyronine, Triomet, specific activity 28.4 mc/mg, and ^{131}I-labeled L-thyroxine, specific activity 35.6 mc/mg, were obtained from Abbott Radio-Pharmaceuticals, Oak Ridge, Tennessee. A Nuclear-Chicago automatic well counter was used to count thyroid and serum samples.

In distribution studies, entire organs were placed in 2-oz disposable plastic medication cups and counted in a holder designed to maintain a constant optimum geometric position in a Packard Armac small animal counter.

External counts for thyroid release experiments employed a Nuclear-Chicago probe and scaler. A specially designed holder was used to maintain a constant geometric relationship between animal thyroid position and the probe; this gave a precision of ±2% between consecutive 1-min counting periods.

The ^{131}I-T_3 uptake studies in serum were performed using a commercial resin sponge kit, Trisorb (Abbott). L-Thyroxine was obtained commercially from Z. D. Gilman, Inc. Thyroid stimulating hormone, Thytropar (Armour), was used for TSH-stimulated release and uptake of ^{131}I. An assay standard of TSH (NIH-TSH-S2) ovine was obtained from the National Institutes of Health, Endocrine Study Section, for use in the McKenzie mouse assay.

Total unbound circulating thyroxine was determined on pooled sera using the resin dialysis method of Ingbar (11). Serum PBI was estimated by an automated alkaline ash method at the First U. S. Army Medical Laboratory, Fort Meade, Maryland.

To determine protein bound radioactivity, whole blood was precipitated with 30% trichloroacetic acid (TCA); the precipitate was washed twice with TCA and then counted. Histologic study of thyroids from infected and healthy rats was performed after formalin fixation and hematoxylin-eosin staining.

Groups of data were analyzed statistically and recorded as the mean $\pm 95\%$ confidence limits. Differences between groups were determined by the *t* test (12) and expressed with a probability value, p.

Results

I. *Thyroidal ^{131}I uptake*

a. *Rat uptake.* Radioactive iodine, 0.5 μc, was administered intravenously (iv) via the dorsal penile vein to 130 to 180 g control rats and to rats infected 40 hours earlier. Ten rats from each group were sacrificed at intervals after injection; their thyroid glands were dissected free and counted. These intervals were selected to permit comparison of uptake *vs.* square root of time as shown in Fig. 1. The usual linear relationship so obtained permitted easier differentiation of early changes in thyroidal uptake (13).

Normal animals exhibited a linear increase in thyroidal radioiodine for the first nine hours. The maximum uptake reached $17.7 \pm 1.0\%$ by 15.5 hours. Thyroidal ^{131}I content showed an appreciable fall by 25 hours. Both the absolute uptake and the timing of uptake duplicated the patterns described for the rat by Pitt Rivers (14).

Infected animals also showed a linear increase in the thyroidal ^{131}I uptake, but, at every time period studied, uptake was less than in the healthy animals, reaching a significant ($p < 0.001$) difference by four hours. The difference between the uptake curves in healthy and infected rats is compatible with depression of thyroidal uptake during infection. At 25 hours, the thyroidal ^{131}I did not begin to fall in infected rats, suggesting that the usual release of hormonal iodide was also delayed.

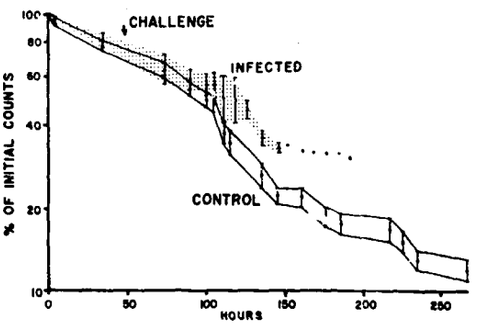

FIG. 2. Influence of pneumoccocal septicemia on thyroidal ^{131}I disappearance in the rat. Data are plotted as the mean $\pm 95\%$ C.L. of 20 control and 15 rats infected 48 hr after beginning measurements of thyroidal ^{131}I release. All but one of the infected animals had succumbed by 150 hr.

b. *Mouse and guinea pig uptake.* Thyroidal uptake of intraperitoneally administered ^{131}I was determined as described above in 240 mice, half of which had been infected 17 hours earlier. The normal animals showed a linear increase in thyroidal radioiodide uptake for nine hours, followed by a rapid fall, suggesting release (Fig. 1). Uptake in infected animals was similar for nine hours but did not fall. At 15.5 hours the infected animals retained slightly but significantly ($p < 0.05$) more thyroidal activity than did the normals. The submaxillary gland uptake of iodide was similar in infected and control groups and appeared to be related to plasma iodide, as shown by others (15). The thyroidal uptake of intravenously injected ^{131}I was similar in infected and control guinea pigs. As reported by others (16), uptake in this species was low,

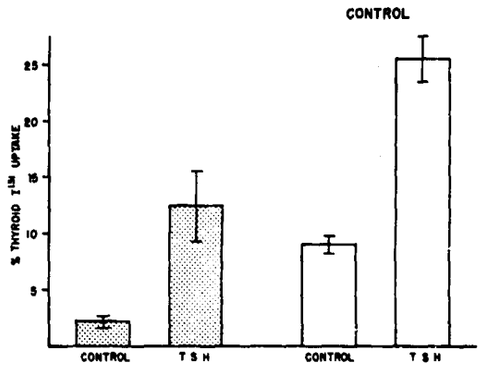

FIG. 3. TSH-stimulated thyroidal ^{131}I uptake in the infected rat. The data shown represent the mean ±95% C.L. thyroidal uptake values with 10 rats/group (see text).

and reached a peak of 6% at 49 hours. Although the infection proved lethal for the mouse, guinea pig and rat, thyroidal iodine uptake was suppressed only in the rat. This suggests that the depression of thyroidal uptake during infection might be a species-specific phenomenon.

II. *Thyroidal ^{131}I release.* Forty μc of ^{131}I was injected intraperitoneally into 35 healthy rats, and thyroidal radioactivity was determined by external counting. Values, corrected for isotope decay, were expressed as a per cent of each animal's 24-hour count.

Forty-eight hours after receiving the radioactive iodide, 15 rats were infected. Results are shown in Fig. 2. As the infected animals became sicker, the disappearance curve became less steep when compared with that of the normals, reaching significance ($p<0.01$) by 103 hours. These results confirmed the studies of Reichlin and Glaser (3), who showed slower and greater variability of thyroidal ^{131}I release curves in the infected animals.

III. *Influence of TSH on thyroidal uptake.* To determine if changes in thyroidal ^{131}I uptake could be related to inability of the gland to respond fully to TSH, a Latin Square experimental design was employed. Forty rats weighing 200 to 250 g were divided into two groups of 20 each. One group was infected 24 hours before the experiment. Both groups were then divided in half. Rats from one subgroup of infected and one subgroup of controls were each given 450 mU (9 mg) TSH subcutaneously at six-hour intervals for a total 24-hour dose of 1800 mU (36 mg). This amount of TSH has been shown to produce a physiologic response in hypophysectomized rats (17). Two hours after the last dose of TSH, all rats were given ^{131}I iv. Four hours later all animals were sacrified and the thyroid glands were counted.

The results are shown in Fig. 3. The ^{131}I uptake at four hours in the infected group was 2.2 ±0.4%, significantly ($p<0.001$) less than that of the healthy group, in which the uptake was 9.0 ±0.6%. After TSH, the uptake in the infected group rose to 12.5 ±3.2%, still significantly ($p<0.001$) less than the post-TSH value of 25.4 ±1.4% in the noninfected group. When the increased uptake after TSH administration was expressed as a percentage of the initial value, infected rats appeared to be more sensitive. Such an interpretation seems unduly weighted by the extremely low initial uptake of the infected animals; based upon the absolute amount of ^{131}I accumulated following TSH stimulation, the infected animal had a significantly depressed ($p<0.01$) increment of rise.

IV. *Influence of TSH on thyroidal ^{131}I release.* Because the ability of TSH to release ^{131}I from the thyroid gland is commonly thought to be a more sensitive indication of the response of the thyroid to TSH than the stimulation of uptake, the following experiment was designed.

Six days before TSH administration, 40 rats were given ^{131}I intraperitoneally. Five hours later 20 μg L-thyroxine was given subcutaneously and 1 grain of desiccated thyroid was added to each 100 ml of drinking water thereafter. Five days before TSH all rats received an additional 10 μg of

L-thyroxine subcutaneously. Two days before TSH administration half the group were infected. Control blood, 0.2 ml, was obtained from the retro-orbital venous plexus and 10 mU (0.02 mg) of TSH was given via the dorsal penile vein. Two hours later a second orbital bleeding was performed. Pre- and post-TSH blood radioactivity was compared.

Results are shown in Fig. 4. Prior to TSH, initial radioactivity in the blood of infected animals was significantly less ($p < 0.0001$) than in controls. Following TSH, blood radioactivity in the infected animals rose only to the basal level of pre-TSH control animals, a degree of release far below ($p < 0.0001$) that seen after TSH administration to normal rats. Although the increase of blood radioactivity, expressed as a percentage of the very low initial value, would at first glance suggest heightened TSH sensitivity of the gland during infection, the actual release of thyroidal ^{131}I was greater ($p < 0.002$) in the normal gland.

To establish whether the observed difference in release might result from a lower ^{131}I content of thyroid glands in infected animals, the entire experiment was repeated in an additional 20 infected and 20 control rats up to the point at which TSH was given. No significant difference could be detected in body weight, thyroid gland weight or in thyroidal ^{131}I. The blood radioactivity of the infected animals was again significantly ($p < 0.002$) below that of control animals, with values comparable to those obtained for each group in the earlier experiment. This observation gave further support to the interpretation that TSH failed to elicit normal release during infection from thyroids of normal weight and ^{131}I content.

In another separate experiment, sections of thyroid glands from five healthy animals and ten animals infected with pneumococcus 44 and 60 hours earlier were studied histologically. No difference was noted in acinar cell height, colloid content or interstitial tissue of thyroids from the infected animals when compared with healthy controls.

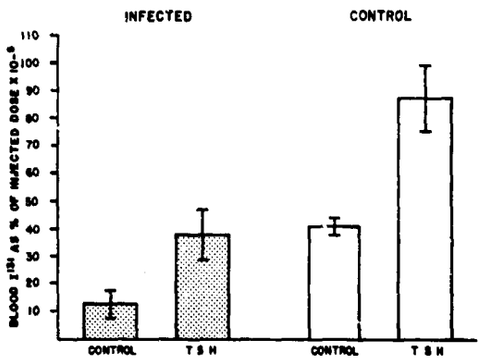

FIG. 4. TSH-stimulated thyroidal ^{131}I release in the infected rat. The data shown represent the mean ±95% C.L. of blood radioactivity before and 2 hr after TSH administration to 20 control and 20 infected rats.

These combined data suggest that the depression of thyroidal uptake and release cannot be explained by a lack of TSH alone but that in infected animals there is a decreased responsiveness of the thyroid gland to TSH. This decreased responsiveness is apparently not related to changes in gland weight or histologic appearance.

V. *Assay of plasma TSH.* The bio-assay of McKenzie (18), as modified by Yamazaki *et al.* (19), was employed to quantitate serum TSH concentration in infected and healthy animals. The NIH standard, NIH-TSH-S2 Ovine, was employed to define a dose response curve, with groups of five mice at each point. Both standard TSH and unknown serum to be assayed were injected in 0.2 ml amounts via the dorsal penile vein. Pooled serum from groups of infected and noninfected rats yielded estimates of TSH concentration of 0.04 ± 0.02 and 0.03 ± 0.02 mU/ml, respectively, a difference of no significance. These concentrations fell within the linear portion of the dose response curve, at least 10-fold above the lower limits of useful sensitivity. Thus, no significant difference appeared to exist in

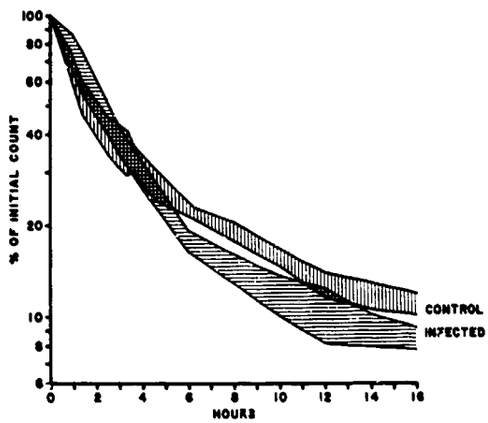

FIG. 5. Disappearance of T_4 from the blood. The data shown represent the mean ±95% C.L. of protein precipitable radioactivity.

TSH concentration between infected and healthy rats when the McKenzie mouse bio-assay was used.

VI. *Disappearance of T_4 from the blood.* To determine if infection altered the rate of disappearance of thyroxine from the blood, ^{131}I-T_4 was administered via the penile vein in a dose of 8 μc (0.2 μg) to ten control rats weighing from 195 to 250 g and to 15 rats of a similar weight which had been infected 40 hours earlier. Orbital blood, 0.2 ml, was then obtained from each rat at 30- to 40-minute intervals for four hours. The blood was washed into 30% TCA; the resulting precipitate was rewashed twice with 10% TCA and then counted. These counts were plotted on semilogarithmic paper, extrapolated to zero time, and expressed as a per cent of the zero time counts. From a second series of similarly treated rats, six orbital bleedings were obtained at hourly intervals. A third series of rats were treated in a similar manner but the experiment was begun 38 hours after infection and bleedings were obtained 6, 8, 10, 12, 14 and 16 hours after injection. All points were corrected to the same standard and plotted on semilogarithmic paper as a per cent of initial counts *vs.* time in hours. The resulting graph showed a curvilinear disappearance of protein precipitable radioactivity (Fig. 5). The curve could be analyzed arbitrarily in three portions. The first portion showed a rapid initial disappearance phase of approximately four to six hours' duration with $t_{\frac{1}{2}}$ of two hours. This portion may represent intravascular, extravascular, and possibly intracellular distribution. The curves for the control and infected animals then became divergent between four and 12 hours. The slope of the curve for infected animals was significantly steeper than that for the control animals with a half disappearance time of 7.6 ± 1.1 and 9.5 ± 1.2 hours, respectively ($p < 0.01$). This portion of the curve may be influenced by an alteration in the fraction of thyroxine bound to plasma proteins. The decreased half disappearance time during infection may reflect a decrease in thyroxine binding by serum proteins. The third portion of the curve, from 12 to 16 hours, flattened in both groups and may represent thyroidal secretion of ^{131}I-labeled hormone formed from recirculated ^{131}I.

VII. *Disappearance of T_3 from the blood.* To determine if infection altered the rate of disappearance of T_3 from the blood, ^{131}I-T_3 was administered via the penile vein in a

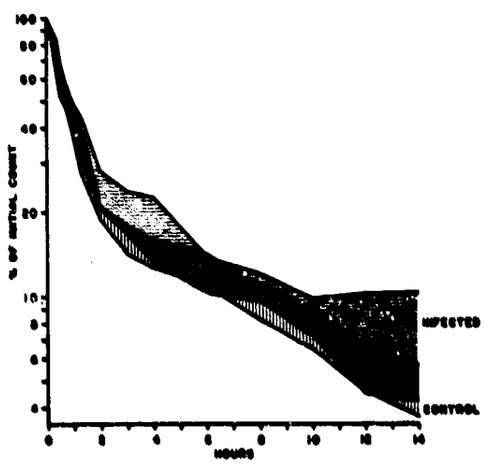

FIG. 6. Disappearance of T_3 from the blood. The data shown represent the mean ±95% C.L. of protein precipitable radioactivity.

TABLE 1. Alterations in thyroid hormones in serum and in their calculated kinetics in the infected rat

	Infected	Control healthy	Control starved
$t_{\frac{1}{2}}$ T_4	7.55 ±1.14 hr (12)	9.47 ±1.15 hr (8)	
PBI	2.1 ±0.8 μg/100 ml (10)	4.9 ±0.5 μg/100 ml (9)	2.3 ±0.8 μg/100 ml (10)
T_4 fractional turnover	9.17%/hr	7.31 %/hr	
Extrathyroidal iodine pool	0.490 μg	1.06 μg	
Degradation rate	.0449 μg/hr	.0772 μg/hr	
Total iodine space	23.4 ml	22.0 ml	
T_3 resin uptake	76.5 ±1.9% (10)	66.2 ±1.6% (10)	64.8 ±3.6% (11)
% unbound T_4	0.37% (2)	0.27% (4)	
Concentration of unbound T_4	11.9 mμg/100 ml	20.3 mμg/100 ml	

No. of rats in parentheses.

dose of 8 μc (0.3 μg) to three groups of ten healthy and 15 rats infected 40 hours earlier. Time intervals, method of sample preparation, and plotting were the same as in the T_4 study. The disappearance of T_3 was curvilinear (Fig. 6). The initial period of rapid disappearance lasted approximately two hours. After two hours the rate of disappearance slowed similarly in both infected and control rats. The more rapid disappearance of T_3 from blood may result from its lower binding affinity for serum proteins. The weaker binding of T_3 may explain its unaltered disappearance pattern after subtle alterations in serum protein binding. In a study of ^{131}I-T_4 disappearance from the blood of humans during typhoid fever no differences from the normal were demonstrated (20).

VIII. *Status of hormones in serum.* To characterize further the apparent depression of thyroid function, the PBI was determined on healthy rats and rats infected 44 hours earlier. As shown in Table 1, a significant ($p < 0.001$) fall was present in the infected rats. Additional control rats starved for 40 hours but allowed water *ad lib.* showed an equivalent depression. This confirmed the earlier work of Reichlin (3, 21) and indicated that starvation could account in part for the fall in PBI.

To determine if the differences in PBI and in rates of T_4 disappearance from blood might be related to a fall in the serum thyroxine binding, the T_3 resin uptake was measured on serum from rats infected 44 hours earlier and healthy controls. The significant increase ($p < 0.0001$) shown in Table 1 for the infected rats gave evidence for a decrease in serum thyroxine binding during infection. A failure of the T_3 resin uptake to change from normal in rats starved for nine days, with a 16.4% fall in body weight, suggested that a starvation-induced fall in PBI was not accompanied by alterations in the binding proteins.

A fall in serum thyroxine binding capacity should be accompanied by an increased percentage of unbound thyroxine. Using the resin dialysis method of Ingbar *et al.* (11), the per cent of unbound thyroxine was measured on pooled serum from groups of healthy rats and from groups infected 44 hours earlier. Values of unbound thyroxine of 0.35 and 0.37% in infected serum pools exceeded the range (0.24–0.28%) of serum pools from normal rats. Despite the increased percentage of unbound thyroxine in serum, the magnitude of the depression of PBI was sufficiently large to produce an absolute decrease in the concentration of serum free thyroxine (Table 1).

Using T_4 $t_{\frac{1}{2}}$ and PBI data, iodine kinetics during infection were estimated according to the method of Sterling and Chodos (22) (see Table 1). The shorter $t_{\frac{1}{2}}$ in the infected group gave an increased fractional turnover of protein precipitable radioactivity. The increased fractional

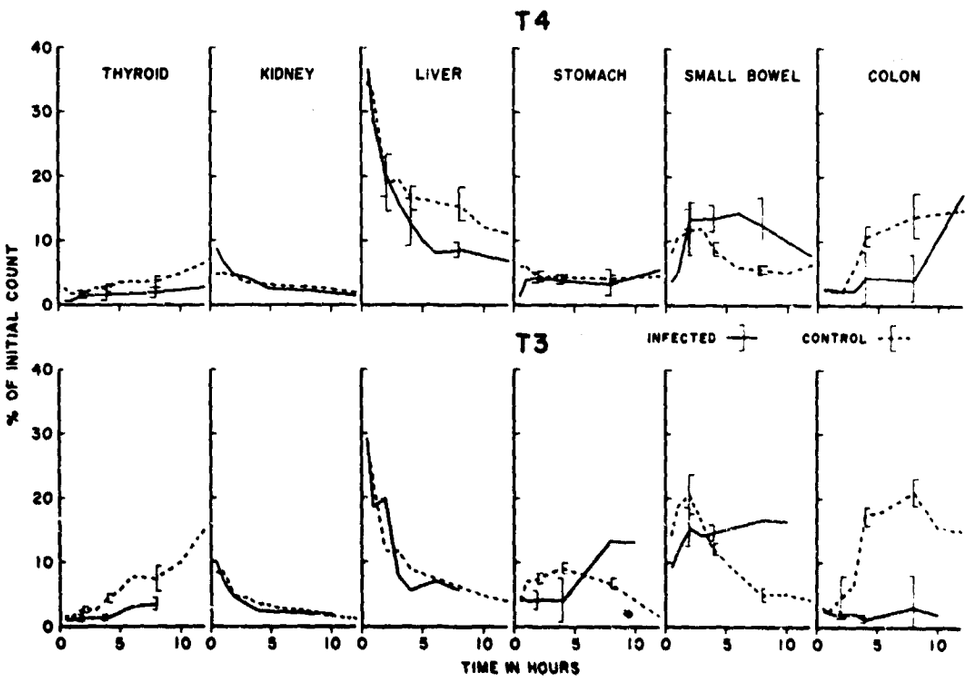

FIG. 7. Distribution of radioactivity after T_3 or T_4 administration to infected rats. Mean ±95% C.L. data are shown for groups of 6 animals sacrificed at 2, 4 and 8 hr following hormone administration. Other points represent single animals (see text). Infected rats are shown by closed circles and solid lines; controls by open circles and dashed lines.

turnover with a fall in PBI resulted in a decreased extrathyrcidal iodine pool and a decreased rate of T_4 degradation. Despite a probable lack of steady state conditions during the course of acute infection, the derived values of an increased fractional turnover imply a decreased serum protein binding of thyroxine (23). The absolute disposal rate of thyroxine has been postulated by Robbins and Rall to be proportional to the concentration of unbound thyroxine in the serum (23). A fall in the absolute disposal of T_4 (degradation rate) in the infected rat gave a theoretic indication for a diminished concentration of circulating unbound thyroxine. This was confirmed by direct measurement, although too few pools were examined to permit appropriate statistical analysis.

IX. *Organ distribution of thyroid hormone.* To determine if infection altered the distribution of thyroid hormones throughout the body, radioactivity of various organs was measured following serial sacrifice after the administration of ^{131}I-T_4 or ^{131}I-T_3 to healthy and infected rats. Because the major changes in the pattern of organ radioactivity occurred within 12 hours of iv administration, this period was studied.

Groups of control and 40-hour infected rats were given either 1 μc (0.03 μg) of ^{131}I-T_4 or 1 μc (0.035 μg) of ^{131}I-T_3 by iv injection. Immediately after injection total body radioactivity was measured for the initial 100% value of each animal. Food and water were then removed. Single rats from each group were sacrificed frequently, and groups of six animals were also sacrificed 2, 4 and 8 hours after hormone injection. Many infected animals were moribund by eight hours.

Immediately after sacrifice total body counts were determined. Whole organ radioactivities of the thyroid, liver, kidneys, stomach, small bowel and colon (including their contents) were measured and expressed as a percentage of the initial total body count.

Healthy and infected animals in the T_4 group retained 91 and 97%, respectively, of initial radioactivity after eight hours, while similar values in the T_3 groups were 79 and 85%.

Fig. 7 portrays graphically the organ distribution of radioactivity in the infected and control animals which received T_4 or T_3. Findings after ^{131}I-T_4 in control animals were similar to those reported by earlier workers (24).

Thyroidal accumulation appeared to be an indirect estimation of hormone deiodination in the control animals; this averaged 4.0 and 7.2%, respectively, eight hours after T_4 or T_3 administration. The thyroidal uptake was significantly less in infected animals which received either hormone.

The over-all patterns of accumulation and disappearance of radioactivity in the stomach, small bowel and colon of control animals was similar after T_4 and T_3 administration, with the exception that values were consistently and significantly higher after T_3, undoubtedly as a result of its more rapid deiodination. In infected animals the normal accumulation of small bowel radioactivity was observed after each hormone, but the bolus of radioactivity then failed to progress into the colon. This slowed movement of accumulated radioactivity in the gastro-intestinal tract following infection could be explained simply on the basis of altered bowel motility. This would also account for the slightly higher retention of total body radioactivity in infected animals at the time of eight-hour sacrifice.

The disappearance of hepatic radioactivity was curvilinear, and on semilogarithmic plot calculations based on the four- to 12-hour portion of the curves revealed half disappearance times for T_4 and T_3 of 14.0 and 6.3 hours, respectively, in control animals. The hepatic $t_{\frac{1}{2}}$ for T_3 radioactivity was not appreciably altered during infection, but after T_4 a significant ($p < 0.01$) shortening was present in the infected rats. These differences seemed analogous to the more rapid disappearance of T_4 from blood during a similar period of time in infected animals.

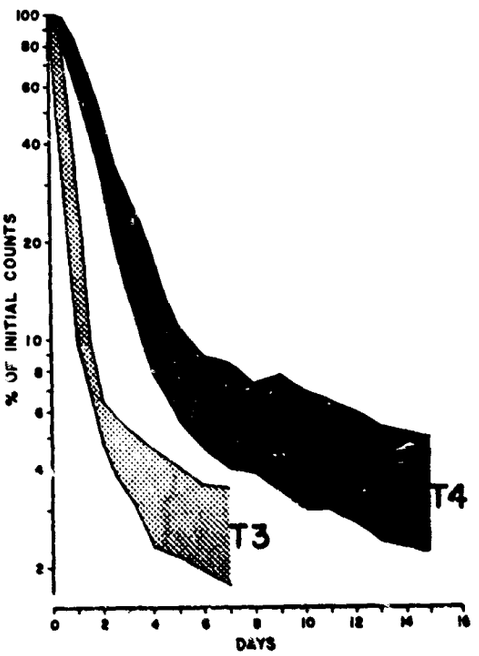

FIG. 8. Disappearance of total body radioactivity following administration of ^{131}I-T_4 or ^{131}I-T_3 to rats. The range of average values of 6 groups of normal rats given T_4 and 5 groups of normal rats given T_3 are shown. Groups of infected rats, studied concomitantly, fall within these ranges.

Radioactivity within the kidney diminished with time in a curvilinear pattern after either T_4 or T_3; no differences were evident in infected rats.

X. *Total body disappearance of hormonal* ^{131}I. Because significant differences were observed in disappearance of T_4 from the blood and various organs of the infected rat, an experiment was designed to determine the net effect of these differences on

total body disappearance over an extended period after iv administration of labeled T_4 and T_3. To reduce the recirculation of ^{131}I released from injected hormone, Lugol's solution was added to the drinking water. In various studies infection was initiated at different intervals before, at the time of, or after hormone administration.

The disappearance of whole body radioactivity from blocked rats given ^{131}I-T_4 followed the pattern shown in Fig. 8. Initially there was a lag phase, which was probably associated with the movement of fecal radioactivity through the gut. Subsequent loss of whole body radioactivity was then curvilinear when plotted on semilogarithmic paper. A rapid phase (uncorrected for later losses) lasted approximately four days ($t_{\frac{1}{2}}$, 1.0 day). Thereafter, isotope was lost at a slower rate with a $t_{\frac{1}{2}}$ averaging 13.8 days. Disappearance after T_3 was similar in general pattern to T_4 but considerably more rapid. No detectable differences in these patterns were induced by the development of infection. Thus, any alterations in the peripheral thyroid hormone physiology noted with infection were too subtle to be detected by the technique of total body counting.

Discussion

These studies were designed to explore the impact of infection on thyroid physiology. Our observations surveyed many areas not previously studied during infection and, in addition, supported the findings of earlier investigators in this field (2, 3). In the rat model employed, infection was accompanied by slight but significant changes in almost every parameter studied: decreased thyroid uptake and release, depressed thyroidal responsiveness to TSH, more rapid T_4 disappearance from blood, associated changes in T_4 binding, turnover and pool size; and finally, altered concentrations of thyroid hormones in several major organs. In contrast, the serum concentration of TSH and the disappearance of T_3 from blood did not appear to change. It seemed possible that these many diverse changes in thyroid physiology during infection could be explained by certain fundamental alterations. In this regard three changes seemed to be of importance: 1) an intrinsic depression of the thyroid gland, 2) a failure of TSH response, and 3) a diminished binding of circulating thyroidal hormones by serum proteins. Other factors, such as altered gut motility, played a lesser role.

Intrinsic depression of thyroid gland function. The changes in thyroidal function during pneumococcal infection in rats included a depression of ^{131}I uptake, a delay in hormone release, and a lessened responsiveness in both functions to exogenous TSH. Similar changes in ^{131}I uptake in the rat have been observed during a number of stresses (6, 9). Inhibition of ^{131}I release from the thyroid of the rat has been observed following bacterial toxin injection (25), as well as infection itself (3). Whether these changes are primary in the thyroid gland, secondary to a suppression of TSH production by the pituitary, or result from generalized factors such as starvation is undetermined. D'Angelo (4) found a depressed serum TSH level which paralleled thyroid follicle involution in rats starved for four to eight days. These workers reversed the starvation-associated histologic alterations by administering TSH, and concluded that, in starvation, a depression of TSH was the cause of the observed thyroidal changes. On the other hand, Badrick and Brimblecombe (5) hypophysectomized rats, and subsequently noted a further suppression of ^{131}I uptake by the thyroids of these animals following the stress of electric shock. They concluded that this stress-induced inhibition of the gland was independent of TSH. Reichlin and Glaser (3) described a delay in thyroidal ^{131}I release in infected rats. Pair fed controls also showed a delay in ^{131}I release, as did a group of infected animals in which dietary intake was maintained by tube feedings.

The present studies of thyroidal response

to TSH brought new information to bear on the problem and indicated that, although stimulation could be elicited by TSH in the infected rat, it was less than normal. The ability of the thyroid gland to respond to exogenous TSH administered seven days after pituitectomy (17) was greater than that observed by us with comparable doses of TSH in infected rats. A possible short-term lack of TSH stimulation during infection thus seems an unlikely explanation for failure of the gland to respond normally.

Since TSH is believed to stimulate all levels of thyroidal hormone synthesis from iodide trapping to hormone release, and since these two specific functions were depressed either with or without exogenous TSH, it is likely that the defect of the rat thyroid in generalized infection is a biochemical one. Lack of change in thyroidal weight or histologic appearance in the present study supports this concept. The nature of such a postulated defect has yet to be identified, but may involve a deficient energy production within thyroid cells, local metabolic or vascular factors (3).

Role of the pituitary. Depressed pituitary TSH output during infection has been postulated by Reichlin and Glaser and explained in part on the basis of starvation or stress of infection (3). Our results indicated that TSH concentrations in infected animals were similar to those of normal controls; the sensitivity of the bio-assay employed permitted detection of grossly high or low values, although the precision did not allow identification of possible subtle changes from normal. Failure of TSH to rise in spite of the marked fall in unbound circulating thyroxine during infection implied that pituitary responsiveness was decreased. Whether pituitary suppression was primary or secondary to a deficiency of hypothalamic thyrotrophin releasing factor (TRF) remains conjectural. The hypothalamic temperature regulating center and the thyroid regulating area have been shown to overlap (26); perhaps fever during infection interfered with TRF production or release (26).

Role of peripheral transport. The increased fractional turnover of thyroxine, compatible with a decrease in serum thyroxine binding during infection in the rat, is analogous to similar observations in the human. An increased turnover of thyroxine in leukemia and in fever associated with urinary tract infection was observed by Sterling and Chodos (22). This observation was confirmed in studies of patients with acute bacterial pneumonia (27) and following major surgical procedures (28). An increased fractional turnover rate of thyroxine in the human thus appears to be a stress-related response (29). Such an increase is compatible with a fall in serum protein binding with an increase in the dialyzable fraction of serum thyroxine shown in the infected rat as well as in the infected human (30). In a variety of nonthyroidal illnesses in the human a decrease in the binding capacity of TBPA has been detected (30, 31). These observations, confirmed and extended in a study of postoperative patients (32), suggested that the decrease in TBPA thyroxine binding capacity might play a role in regulating the concentration of unbound thyroxine in response to physiologic needs (32). *In vitro* temperature elevation within clinical ranges has been shown to increase the fraction of unbound thyroxine in serum (33). A similar effect in the febrile patient, however, may not be demonstrable when the serum is studied at 37 C *in vitro* (34). The role of fever was not investigated in the rat, but it could have been a factor in the alteration of binding (33, 34). Although TBG has not been shown to change during acute stress, a decreased synthesis of TBPA may occur in acute or chronic illness (35).

The above-mentioned studies suggest that, in the human, surgical stress, infection or pyrogen administration results in a decrease in the binding capacity of TBPA

with an increase in the unbound fraction of thyroxine. If alterations in the peripheral metabolism of T_4 in the infected rat were indeed related to decreased serum protein binding, the peripheral metabolism of a more weakly bound thyroid hormone such as T_3 might be affected less by a change related to binding proteins. In the infected rat, the disappearance rate of T_3 from the blood was unchanged, while that of T_4 was increased; the hepatic disappearance rate of T_3 was similar to normal, while that of T_4 was increased. These differences in the peripheral metabolism of T_3 and T_4 add weight to the concept that a change in protein binding of thyroxine plays a role in altered thyroid physiology. In contrast to infection, starvation *per se* did not appear to influence protein binding. In the rat, various stresses, including exposure to cold, violent exercise, anoxia, adrenalin, nephrectomy, killed typhoid bacilli and starvation (10, 21) have been shown to decrease serum PBI. To this list may be added the stress of infection. Such a stress-related fall in PBI has also been shown in thyroidectomized animals (10), suggesting that an alteration in the protein binding of T_4 is not unique for infection.

Other factors. Infection acted as a profound depressor of gut motility, with the result that the major bolus of radioiodine formed by secretion of unbound iodide in the stomach and small bowel and conjugated hormone via the biliary tree had not reached the colon in the infected animals by eight hours. This observation may account for the slight delay in total body disappearance of radioiodide from the infected animals. The significantly higher concentration of radioactivity in the intestine of healthy rats given T_3 may be compatible with an increase either in iodide excretion secondary to deiodination of a hormone that is weakly bound to plasma proteins, or in excretion of the intact hormone or one of its cogeners. Changes in intra- and extracellular distribution of T_4 and T_3 during infection were not studied, and their contributions to altered peripheral metabolism remain unknown.

Species differences in the response of thyroidal function to infection were apparent in the rat, mouse and guinea pig. Since the thyroidal ^{131}I uptake of the mouse infected with *C. immitus* was depressed (2) in contrast to infection with pneumococcus type IA-5, the reaction to the stress of infection may be not only species specific, but also organism specific.

Using the model of the Dunning Fisher rat and *D. pneumoniae* we could demonstrate no alteration that would differentiate the specific stress of this infection from any other nonspecific stress. Such differences, should they exist, may do so at an intracellular level, not directly studied in this report.

References

1. Shick, B., P. Cohen, and I. Beck, *Amer J Dis Child* **31:** 228, 1926.
2. Sternberg, T. H., V. D. Newcomer, C. G. Steffen, M. Fields, and R. L. Libby, *J Invest Derm* **24:** 397, 1955.
3. Reichlin, S., and R. J. Glaser, *J Exp Med* **107:** 219, 1958.
4. D'Angelo, S. A., *Endocrinology* **48:** 341, 1951.
5. Badrick, F. E., R. W. Brimblecombe, J. M. Reiss, and M. Reiss, *J Endocr* **11:** 305, 1954.
6. Bogoroch, R., and P. Timiras, *Endocrinology* **49:** 548, 1951.
7. Catz, B., I. El Rawi, and E. Geiger, *Amer J Physiol* **172:** 291, 1953.
8. Van Middlesworth, L., and M. M. Berry, *Amer J Physiol* **167:** 576, 1951.
9. Williams, R. H., H. Jaffe, and C. Kemp, *Amer J Physiol* **159:** 291, 1949.
10. Bondy, P. K., and M. A. Hagewood, *Proc Soc Exp Biol Med* **81:** 328, 1952.
11. Ingbar, S. H., L. E. Braverman, N. A. Dawber, and G. Y. Lee, *J Clin Invest* **44:** 1679, 1965.
12. Batson, H. C., An Introduction to Statistics in the Medical Sciences, Burgess Publ. Co., Minneapolis, Minn., 1956, p. 16.
13. Stanley, M. M., and E. B. Astwood, *Endocrinology* **41:** 66, 1947.
14. Pitt-Rivers, R., *Biochem J* **82:** 108, 1962.
15. Myant, N. B., *Ann NY Acad Sci* **85:** 208, 1960.
16. Schindler, W. J., and C. Fortier, *Canad J Biochem Physiol* **40:** 1641, 1962.
17. Taurog, A., W. Tong, and I. L. Chaikoff, *Endocrinology* **62:** 664, 1958.
18. McKenzie, J. M., *Endocrinology* **63:** 372, 1958.
19. Yamazaki, E., A. Noguchi, S. Sato, and D. W. Slingerland, *J Clin Endocr* **21:** 1127, 1961.
20. Wiswell, J. G., and V. Coronho, *J Clin Endocr* **22:** 657, 1962.

21. Reichlin, S., *Endocrinology* **60:** 470, 1957.
22. Sterling, K., and R. B. Chodos, *J Clin Invest* **35:** 806, 1956.
23. Robbins, J., and J. E. Rall, *Physiol Rev* **40:** 415, 1960.
24. Albert, A., and R. F. Keating, *Endocrinology* **51:** 427, 1952.
25. Gerwing, J., D. A. Long, and R. Pitt-Rivers, *J Physiol (London)* **144:** 229, 1958.
26. Reichlin, S., *Endocrinology* **66:** 340, 1960.
27. Gregerman, R. I., and N. Solomon, *Clin Res* **12:** 268, 1964.
28. Blomstedt, B., *Acta Chir Scand* **130:** 424, 1965.
29. Ingbar, S. H., *Ann NY Acad Sci* **86:** 440, 1960.
30. Oppenheimer, J. H., R. Squef, M. I. Surks, and H. Hauer, *J Clin Invest* **42:** 1769, 1963.
31. Richards, J. B., J. T. Dowling, and S. H. Ingbar, *J Clin Endocr* **38:** 1035, 1959.
32. Surks, M. I., and J. H. Oppenheimer, *J Clin Endocr* **24:** 794, 1964.
33. Schussler, G. C., and J. E. Plager, *Clin Res* **13:** 248, 1965.
34. Bernstein, G., and J. H. Oppenheimer, *J Clin Endocr* **26:** 195, 1966.
35. Solomon, E. L., K. A. Woeber, H. Purdy, M. Holloway, and S. H. Ingbar, *Clin Res* **13:** 248, 1965.