Alterations in Thyroid Hormone Economy during Acute Infection with *Diplococcus pneumoniae* in the Rhesus Monkey

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ABSTRACT In order to study the alterations in thyroid hormone economy that accompany an acute bacterial infection, rhesus monkeys were inoculated i.v. with a virulent *Diplococcus pneumoniae* culture containing approximately 10^8 organisms per dose. This was found to produce a well-defined febrile illness followed in most instances by spontaneous recovery, thereby permitting sequential observations to be made during progression from the healthy state through acute infection into convalescence. During the acute febrile period of the infection, the clearance of both exogenously labeled L-thyroxine (T_4) and 3,3',5-triiodo-L-thyronine (T_3) from their peripheral pools was accelerated. This alteration was often evident by 8 hr after inoculation with the virulent culture and could not be ascribed to a decrease in extracellular binding. Despite the accelerated hormonal clearance, the concentrations of both endogenously labeled thyroid hormone and stable T_4 in the sera of the surviving monkeys remained essentially unchanged or increased, indicating that hormonal secretion must have increased during this period. During the convalescent period, hormonal clearance was similar to preinfection control values. Leukocytes isolated from blood obtained 6 hr after inoculation with the virulent culture displayed enhanced T_4-deiodinative activity.

INTRODUCTION

The effects of acute infection on thyroid hormone economy are uncertain. Although acute infection has been shown to influence thyroid function and the peripheral metabolism of L-thyroxine (T_4) and 3,3',5-triiodo-L-thyronine (T_3) in several animal species, the results of such studies tend to be conflicting, appearing to vary with the nature of the species examined. In the rat, for example, acute streptococcal and pneumococcal infections appear to result in a depression of thyroid function (1, 2), whereas in man data obtained after treatment of acute pneumococcal pneumonia had been instituted suggest that an increase in thyroid function might accompany this type of stress (3).

We undertook the present study in order to assess in a sequential fashion various aspects of thyroid hormone economy during progression from the healthy state through an acute bacterial infection into convalescence. The rhesus monkey was employed as the animal model because we felt that its response should be most closely representative of that of man. *Diplococcus pneumoniae* was employed as the infecting microorganism.

METHODS

Male rhesus monkeys (*Macaca mulatta*) weighing between 2.1 and 3.7 kg were secured in primate chairs. In the experiment in which it was desired to label the intrathyroidal iodine pool, eight monkeys were fed a low iodine diet.[1] After a 3 wk period of adaptation had elapsed, each monkey was given an i.v. injection of 25 μCi of carrier-free inorganic ^{125}I [2] to label the intrathyroidal iodine pool. 1 ml blood samples were obtained by saphenous venipuncture daily thereafter. 10 days later, each monkey was given an i.v. injection of 10 μCi (approximately 0.3 μg) of ^{131}I-labeled L-thyroxine[3] (T_4-^{131}I) in 1 ml of 1% (w/v) human serum albumin to label the peripheral hormonal pool. After a 5

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[1] Modified SKF monkey diet containing vitamin fortification mix and U.S.P. salt mix (less iodine), obtained from General Biochemicals, Div., North American Mogul Products Co., Chagrin Falls, Ohio.

[2] Obtained from New England Nuclear Corp., Boston, Mass.

[3] Obtained from Abbott Laboratories, Chemical Marketing Div., North Chicago, Ill.

day control period had elapsed, six monkeys were inoculated i.v. with a *Diplococcus pneumoniae* (Type 1-A) culture containing approximately 10^8 virulent organisms per dose, and two were sham-inoculated with an equal volume of normal saline. This was immediately followed by a second i.v. injection of 10 μCi of T_4-^{131}I. 1 ml blood samples were obtained every 8 hr for the first 32 hr after inoculation, and collections were continued daily thereafter. 5 days after inoculation, the surviving monkeys were given a third i.v. injection of 10 μCi of T_4-^{131}I, and collection of blood samples was continued for a further 5 days. Two additional monkeys were employed to assess the effects of a heat-killed *D. pneumoniae* culture on the peripheral metabolism of T_4-^{131}I. Here, the culture was subjected to a temperature of 57.5°C for 30 min, and plating out confirmed the absence of viable organisms. Immediately after inoculation with the heat-killed culture, 10 μCi of T_4-^{131}I was injected i.v., and blood samples were obtained over the next 5 days.

In the experiment in which it was desired to assess concurrently the peripheral metabolism of both T_4 and T_3, four additional monkeys were maintained on a diet which had an iodine content of 1.6 ppm.[4] Two monkeys were inoculated i.v. with the virulent *D. pneumoniae* culture, and this was followed immediately by an i.v. injection of 10 μCi of T_4-^{131}I and 5 μCi (approximately 0.1 μg) of ^{125}I-labeled T_3[5] (T_3-^{125}I). The remaining two monkeys served as controls. 1 ml blood samples were obtained by saphenous venipuncture every 8 hr for the first 48 hr after inoculation and then daily for an additional 3 days.

In all experiments, all injections were given between 1 and 2 p.m., and days or fractions thereof are in relation to this time. Temperature was measured rectally with a thermistor thermometer.

The concentrations in serum of protein-bound ^{125}I (PB^{125}I and T_3-^{125}I) and protein-bound ^{131}I (T_4-^{131}I) were measured as follows. To 250 μl of serum were added 1 drop of 2 M potassium iodide and a few milligrams of thiouracil, and the protein-bound radioactivity was precipitated with cold 20% trichloroacetic acid. The precipitates were washed twice with cold 5% trichloroacetic acid and then dissolved with 2 N NaOH to a standard volume for counting in a well-type scintillation counter. Corrections were made for the contribution of the ^{131}I to the ^{125}I counts in those samples that contained both isotopes. Counting standards were prepared from the injection solutions immediately after administration and, in the case of T_4-^{131}I and T_3-^{125}I, were also subjected to precipitation with trichloroacetic acid after the addition of a small amount of 25% (w/v) human serum albumin. 94–96% of both the labeled T_4 and T_3 was recovered in the precipitate.

After each injection of T_4-^{131}I, the declining concentration of radioactivity in serum during the subsequent 5 day period was plotted against time. By 16 hr after injection, the curve appeared to conform to a single exponential function, indicating that distribution equilibrium of the residual T_4-^{131}I had been attained. The data obtained at 16 hr and thereafter were therefore used to calculate values for the fractional rate of T_4 disappearance. In the infected monkeys, the rate of disappearance of T_4-^{131}I from serum slowed abruptly between 2 and 3 days after inoculation with the culture. Accordingly, values for the fractional disappearance rate were calculated separately from the data obtained during the first 2 days and from the data obtained from days 3 through 5. Values for the volume of T_4 distribution were calculated as the quotient of injected radioactivity and the concentration of radioactivity in serum at the time of injection as obtained by backward extrapolation of the disappearance curve. Correction was always made for the concentration of radioactivity remaining from a previous injection of T_4-^{131}I. In the case of the infected monkeys, values for the volume of distribution during the period from days 3 through 5 after inoculation with the culture were calculated as follows. Since it was not possible to obtain complete urine and stool collections, the residual T_4-^{131}I at the end of the first 2 days was estimated as the product of the values for the calculated volume of distribution during this period and the concentration of T_4-^{131}I in serum on day 2. The volume of distribution during the period from days 3 through 5 was then calculated as the quotient of this value and the value for the concentration of T_4-^{131}I in serum on day 2 derived by backward extrapolation of the disappearance curve for days 3 through 5. The rate of T_4 clearance was calculated as the product of volume of distribution and fractional disappearance rate, and the absolute rate of T_4 disappearance as the product of clearance rate and the concentration of endogenous T_4 in serum.

In the case of T_3, the disappearance curve did not conform to a single exponential function, but slowed progressively with time. Accordingly, the method of "peeling" was employed to derive an estimate of the rate of disappearance of T_3-^{125}I. Owing to the small number of later points and hence the essentially arbitrary nature of the peeling, no attempt was made to calculate kinetic data for T_3 disappearance.

The binding of T_4 in serum was assessed by enriching serum samples with the equivalent of approximately 70 μg of T_4-^{131}I per 100 ml and subjecting them to reverse-flow filter paper electrophoresis in glycine(0.2 M)-acetic acid (0.13 M) buffer at pH 8.6, using a Durrum-type electrophoresis cell (4). Serum samples from each monkey were always subjected to electrophoresis concurrently in a single cell. The distribution of T_4-^{131}I among the binding proteins was quantitated by cutting out the radioactive zones on the filter paper strips with the aid of radioautographs and counting them in a well-type scintillation counter.

The concentration of endogenous T_4 in serum was measured by the binding displacement method of Murphy and Pattee (5).[6] Owing to the small volumes of serum, it was necessary to pool several samples for this determination.

The effects of acute infection in vivo on the deiodination of T_4-^{131}I by leukocytes in vitro was assessed in two experiments. In each experiment, two fresh monkeys were employed of which one was inoculated i.v. with the *D. pneumoniae* culture containing approximately 10^8 virulent organisms per dose and the other with the heat-killed culture. In the one experiment, heparinized blood samples were collected in chilled. tubes on the day before inoculation, immediately before inoculation, 6 hr after inoculation, daily thereafter for 3 days, and on day 6 after inoculation. In the other experiment, heparinized blood samples were obtained immediately before and again 6 hr after inoculation. Immediately after collection, the processing of the blood was begun, and the whole leukocyte isolation procedure was carried out in chilled laboratory ware. The incubation with T_4-^{131}I was begun immediately after the leukocyte isolation had been completed. The samples from both monkeys in each experiment were handled concurrently. Plastic laboratory ware

[4] Monkey chow obtained from Ralston Purina Co., St. Louis, Mo.

[5] Obtained from Abbott Laboratories.

[6] Performed by the Boston Medical Laboratory, Waltham, Mass.

or siliconized glassware were used throughout. The leukocytes were isolated from the blood by the method of Bertino *et al.* (6). Briefly, the blood was sedimented in a dextran–saline solution, and the supernatant layer containing the leukocytes was centrifuged. The erythrocytes remaining in the leukocyte pellet were lysed with cold, distilled water and removed by washing. Finally, the leukocytes were suspended in Krebs-Ringer phosphate buffer at pH 7.4 containing 2 mg of glucose per ml (KRPG). 1 ml of the leukocyte suspensions was added to Erlenmeyer flasks containing 1 ml of KRPG and 50 μl (approximately 0.25 μg) of T_4-^{131}I. Additional flasks containing 2 ml of KRPG and 50 μl of T_4-^{131}I were prepared to serve as tissue-free controls. All flasks were prepared in duplicate. The flasks were incubated at 37°C in 100% oxygen in a metabolic shaker. Preliminary experiments had indicated that plateau values for T_4-^{131}I deiodination by leukocytes are attained by approximately 1 hr. Consequently, in the one experiment in which it was desired to obtain plateau measurements, incubation was allowed to proceed for 2 hr. After incubation, 500 μl of 25% (w/v) human serum albumin containing potassium iodide and thiouracil was added to each flask to stop the reaction. In the other experiment, the rate of T_4-^{131}I deiodination was assessed by withdrawing samples from the incubation medium after 10, 15, and 30 min of incubation and transferring them to tubes containing serum albumin, potassium iodide, and thiouracil. 20 μl from each flask or tube were then subjected to ascending chromatography in No. 1 Whatman filter paper strips in a butanol–acetic acid–water (120:30:50) solvent system (7). The percentage of the total radioactivity present as inorganic iodide and origin material, representing the percentage of T_4-^{131}I deiodinated, was quantitated by cutting out the radioactive zones on the filter paper strips with the aid of radioautographs and counting them in a well-type scintillation counter. These values were corrected for spontaneous deiodination by subtracting from them the corresponding tissue-free control value.

RESULTS

Fig. 1 depicts the results of an experiment conducted in eight monkeys in which the intrathyroidal iodine pool was labeled with inorganic ^{125}I and the peripheral hor-

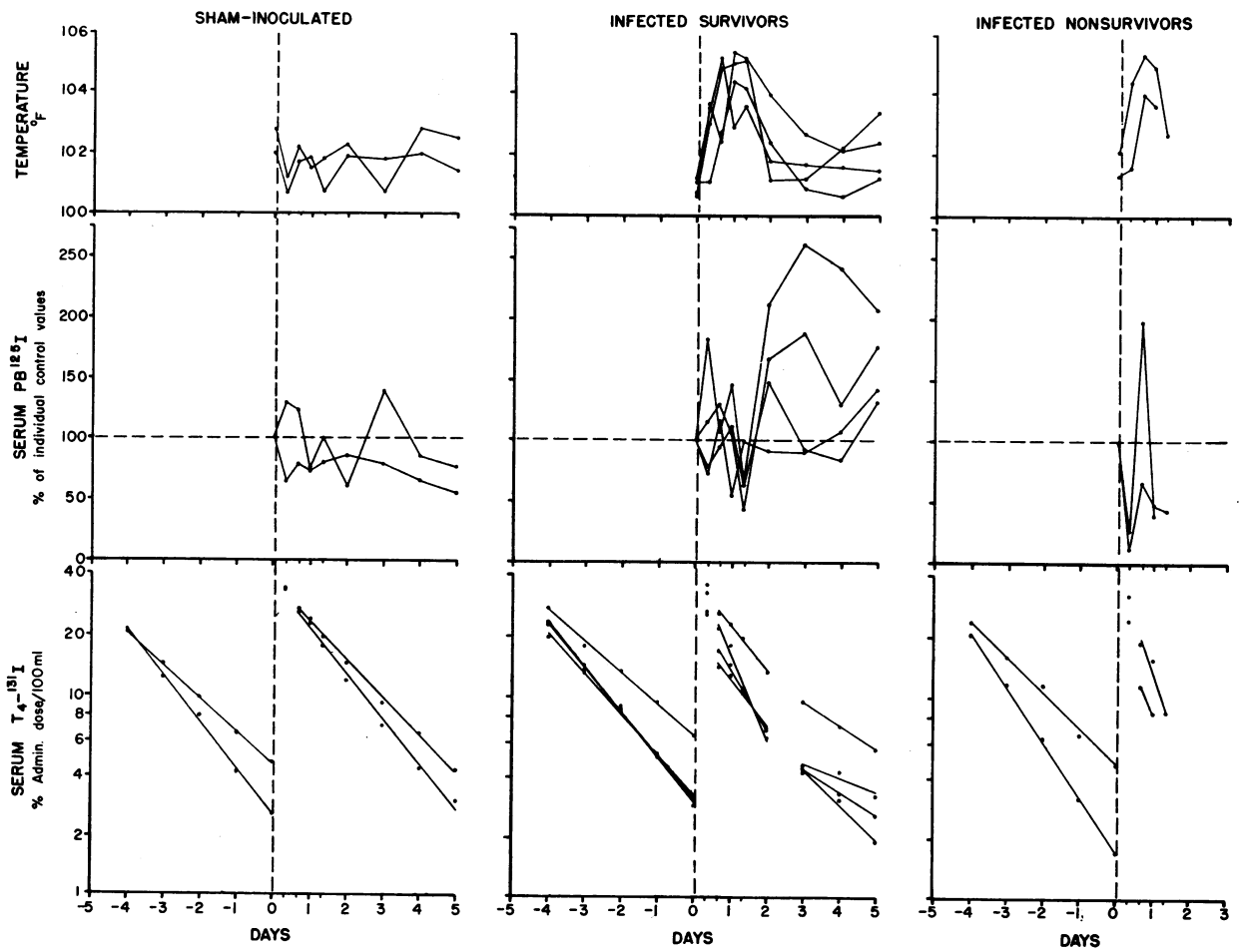

FIGURE 1 The effects of acute infection with *Diplococcus pneumoniae* on the concentration in serum of endogenously synthesized protein-bound ^{125}I ($PB^{125}I$) and on the disappearance from serum of injected ^{131}I-labeled L-thyroxine (T_4-^{131}I). The time of inoculation with the culture or sham-inoculation with normal saline is designated as day 0.

TABLE I

The Effects of Acute Infection with Diplococcus pneumoniae on the Kinetics of Peripheral ^{131}I-Labeled L-Thyroxine (T_4-^{131}I) Metabolism

Monkey No.	Weight	Period*	Volume of T_4 distribution	Fractional T_4 disappearance rate	T_4 clearance rate	Serum T_4	Absolute T_4 disappearance rate
	kg		*ml*	*%/day*	*ml/day*	*μg/100 ml*	*μg/day*
Sham-inoculated with normal saline							
1	2.28	Control	336	38	128	5.5	7.0
		Postinoculation	327	42	137	5.5	7.5
2	2.26	Control	284	53	151		
		Postinoculation	305	51	156		
Infected by inoculation with the virulent culture							
3	2.36	Control	270	35	94	6.0	5.6
		Acute (104.4°F at 24 hr)	310	52	161	7.0	11.3
		Convalescent I	340	26	88	7.5	6.6
		Convalescent II	305	30	92	7.5	6.9
4	2.48	Control	323	45	145	6.0	8.7
		Acute (105.4°F at 24 hr)	554	53	294	6.0	17.6
		Convalescent I	619	40	248	7.0	17.4
		Convalescent II	421	44	185		
5	2.38	Control	260	52	135		
		Acute (105.1°F at 32 hr)	400	70	280		
		Convalescent I	480	26	125		
		Convalescent II	306	37	113		
6	2.44	Control	272	50	136	4.5	6.1
		Acute (105.2°F at 16 hr)	253	98	248	5.8	14.4
		Convalescent I	284	16	45	8.5	3.8
		Convalescent II	333	37	123	6.5	8.0
7	2.44	Control	278	41	114	4.5	5.1
		Acute (105.3°F at 16 hr)	247	119	294	6.0	17.6
8	2.13	Control	260	62	161		
		Acute (104.0°F at 16 hr)	449	105	471		
Inoculated with the heat-killed culture‡							
9	3.67	Postinoculation	341	39	133		
10	3.60	Postinoculation	372	38	141		

* In this column, the term "acute" refers to the first 2 days after inoculation with the virulent culture, and the maximum temperature and its time of occurrence are indicated in parentheses. Convalescent I refers to days 3 through 5 after inoculation. The same injection of T_4-^{131}I was used to assess the kinetics of T_4 metabolism during both these periods. Convalescent II refers to the period following the fresh injection of T_4-^{131}I on day 5 after inoculation.

‡ Control observations prior to inoculation were not obtained in this group. Nevertheless, the values following inoculation are similar to the control values presented here.

monal pool with T_4-^{131}I. The time of inoculation is designated as zero time and is indicated by the dashed vertical lines. As depicted in the upper panels, fever was usually present by 8 hr after inoculation with the culture and lasted for 2–3 days. A moderate neutrophilia was also present during this period in the four monkeys that survived the infection, whereas leukopenia occurred in the two nonsurvivors.[7] This initial period will be termed the acute period, whereas the period from day 3 onwards will be termed the convalescent period. Table I summarizes the data for the kinetics of T_4-^{131}I metabolism in

[7] A necropsy was performed on one of these (monkey No. 7) and revealed pulmonary congestion and edema. Pure cultures of *D. pneumoniae* were obtained from heart blood and from lung and liver tissue.

the individual monkeys and also includes the data obtained in the two monkeys inoculated with the heat-killed culture. The values for the concentration of endogenously synthesized $PB^{125}I$ in the sera of the individual monkeys are presented in Table II.

During the acute febrile period of the illness, the rate of disappearance of T_4-^{131}I from serum, depicted in the lower panels of Fig. 1, was increased in all six infected monkeys relative to their control values (Table I). In four of the six infected monkeys, the calculated volume of T_4 distribution was increased during the acute febrile period (Table I). Consequently, the calculated rate of T_4 clearance was greatly increased, and for the group of six infected monkeys as a whole this increase was significant statistically as judged from the paired t test ($P < 0.01$). These alterations were greater in the two nonsurviving monkeys than in the four infected survivors. Despite the increased rate of T_4 clearance, serum T_4 was increased in three infected monkeys relative to their control values and unchanged in one so that the absolute rate of T_4 disappearance was increased during this period (Table I).

The values for endogenously synthesized $PB^{125}I$ in serum are depicted in the middle panels of Fig. 1 as a per cent of the mean of the values obtained during the control period from days -5 through 0 for each monkey and are also presented in Table II. During the acute febrile period, serum $PB^{125}I$ changed little in two of the infected survivors and increased in the other two despite the increased rate of T_4 clearance. In the two nonsurvivors, serum $PB^{125}I$ decreased in one and fluctuated widely in the other.

During the convalescent period from day 3 onwards when the temperature had returned toward control values, a decrease in the rate of disappearance of T_4-^{131}I from serum was observed (Fig. 1), and the values were consistently less than the control values prior to infection (Table I, convalescent I). On the other hand, the increase in the calculated volume of T_4 distribution persisted, with the result that the calculated rate of T_4 clearance was similar to the preinfection control values (Table I, convalescent I). As depicted in Fig. 2, T_4-^{131}I given as a fresh injection during the convalescent period on day 5 after inoculation with the culture be-

TABLE II

*The Effects of Acute Infection with Diplococcus pneumoniae on the Concentration in Serum of Endogenously Synthesized Protein-Bound ^{125}I ($PB^{125}I$) after the Administration of Inorganic ^{125}I on Day -10**

Monkey No.	Serum $PB^{125}I$ on day													
	−5	−4	−3	−2	−1	0	0.33	0.67	1	1.33	2	3	4	5
	% Admin. dose × 10^3/100 ml													
Sham-inoculated with normal saline														
1	60.2	61.7	68.5	48.4	44.4	55.3	35.4 (63)‡	43.8 (78)	41.0 (73)	44.6 (79)	48.6 (86)	43.8 (78)	36.8 (65)	31.1 (55)
2	21.9	35.9	30.5	35.1	12.1	42.9	38.3 (129)	36.5 (123)	22.2 (75)	29.3 (99)	17.7 (60)	41.2 (139)	25.3 (85)	22.5 (76)
Infected by inoculation with the virulent culture														
3	67.3	55.7	70.9	55.8	59.6	64.9	45.4 (73)	71.5 (115)	33.0 (53)	61.0 (98)	56.3 (90)	54.6 (88)	66.3 (106)	88.2 (141)
4	31.4	11.1	29.3	31.3	32.9	23.0	30.1 (114)	34.2 (129)	27.5 (104)	10.8 (41)	44.1 (166)	49.6 (187)	33.8 (128)	46.6 (176)
5	23.6	28.7	15.9	15.1	14.6	21.7	36.3 (182)	21.1 (106)	28.6 (144)	13.2 (66)	29.3 (147)	18.2 (91)	16.6 (83)	26.1 (131)
6	28.4	21.0	17.4	20.7	32.3	38.8	20.2 (77)	24.5 (93)	29.3 (111)	16.4 (62)	55.7 (211)	68.6 (260)	63.6 (241)	54.4 (206)
7	103.9	115.2	102.4	105.4	70.2	97.9	11.2 (11)	65.2 (66)	46.8 (47)	42.7 (43)	Died			
8	32.0	29.6	17.7	28.0	33.9	35.4	8.1 (28)	58.1 (198)	11.3 (38)	Died				

* The time of inoculation is designated as day 0 and immediately followed the collection of blood for the measurements shown in this column.

‡ The values in parentheses are those depicted in Fig. 1 and are the per cent of the mean of the values during the control period from days -5 through 0 for each monkey.

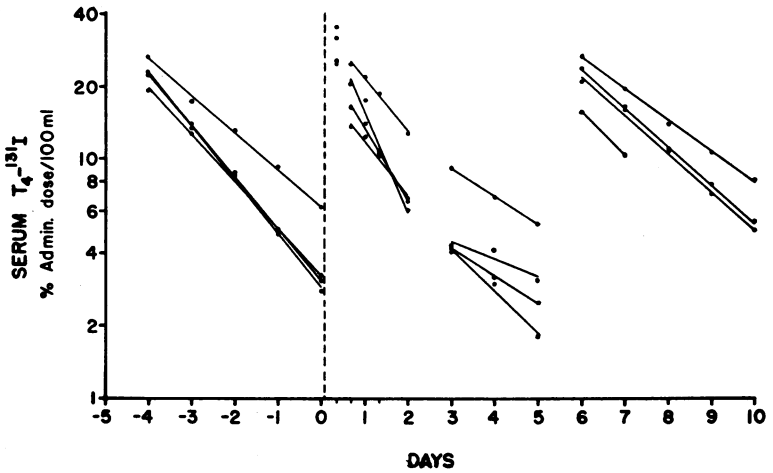

FIGURE 2 The disappearance from serum of ^{131}I-labeled L-thyroxine (T_4-^{131}I) given as a fresh injection on day 5 after inoculation with the culture. The preceding disappearance curves are those depicted in Fig. 1 for the infected survivors.

haved in a similar fashion. Its rate of disappearance from serum was less and its calculated volume of distribution greater than the control values in all four survivors (Table I, convalescent II). Values for serum T_4 in the three survivors in which this determination was obtained were greater than the values in the same monkeys during the preceding acute and control periods (Table I). Distinct increases in serum $PB^{125}I$ were also present during the convalescent period in two of the four survivors (Table II), and the monkey who displayed the greatest increase (monkey No. 6) also had the greatest increase in serum T_4.

No alterations in the distribution of T_4-^{131}I among T_4-binding globulin (TBG), albumin, or T_4-binding prealbumin (TBPA) were observed during the acute period of the illness (Table III). However, on day 6 during the convalescent period, a slight increase in the per cent of T_4 bound by TBG and a corresponding decrease in the per cent bound by TBPA were observed.

No alterations in the kinetics of T_4-^{131}I metabolism, in serum $PB^{125}I$, or in T_4-binding in serum were observed in the two monkeys sham-inoculated with normal saline. Likewise, in the two monkeys inoculated with the heat-killed culture, the values for the kinetics of T_4-^{131}I metabolism were similar to the control values in the other eight monkeys presented in Table I.

Fig. 3 compares the kinetics of T_3-^{125}I and T_4-^{131}I disappearance in two infected monkeys with those in two control monkeys. In the infected monkeys, the duration of the acute febrile period and the alterations in the kinetics of T_4-^{131}I disappearance were the same as those described earlier. In both the control and infected monkeys, the rate of disappearance of T_3-^{125}I from serum decreased progressively with time. Nevertheless, the slope of the straight line derived by the peeling technique was greater in the infected monkeys during the acute period, indicating an increased fractional rate of T_3 disappearance.

Table IV compares the plateau values for in vitro deiodination of T_4-^{131}I by leukocytes from an infected

TABLE III

*The Effects of Acute Infection with Diplococcus pneumoniae on the Binding of ^{131}I-Labeled L-Thyroxine (T_4-^{131}I) by T_4-Binding Globulin (TBG) and T_4-Binding Prealbumin (TBPA) in Serum**

Monkey No.		Day‡				
		−5	−3	0.33	2	6
Sham-inoculated with normal saline						
1	%T_4-TBG	30	32	28	32	31
	%T_4-TBPA	39	38	38	37	35
2	%T_4-TBG	23	18	30	26	23
	%T_4-TBPA	28	26	26	28	28
Infected by inoculation with the virulent culture						
3	%T_4-TBG	27	22	23	26	29
	%T_4-TBPA	41	40	48	46	39
4	%T_4-TBG	19	28	29	31	34
	%T_4-TBPA	45	36	37	37	24
5	%T_4-TBG	22	21	21	28	34
	%T_4-TBPA	49	47	51	43	39
6	%T_4-TBG	31	25	22	23	45
	%T_4-TBPA	41	45	49	37	31

* Serum samples were enriched with the equivalent of approximately 70 μg of T_4-^{131}I per 100 ml.

‡ The time of inoculation is designated as day 0.

monkey with those from a monkey inoculated with the heat-killed culture. It will be noted that before inoculation both monkeys displayed a relative neutropenia, lymphocytes being the preponderant cell form present (this is generally the case in the healthy rhesus monkey). After inoculation with either the virulent or the heat-killed culture, both monkeys developed a neutrophilia. An increase in T_4-^{131}I deiodination was observed with leukocytes obtained 6 hr after inoculation with the virulent culture. This did not occur after inoculation with the heat-killed culture despite the apparently similar alteration in the leukocyte population of peripheral blood. Fig. 4 compares the early rate of T_4-^{131}I deiodination by leukocytes from two additional monkeys of which one was inoculated with the virulent culture and the other with the heat-killed culture. A distinct increase in the rate of deiodination per unit number of leukocytes was observed 6 hr after inoculation with the virulent culture.

DISCUSSION

In the present study, we have attempted to define the alterations in thyroid hormone economy that accompany an acute bacterial infection. When administered intravenously to the rhesus monkey, the *D. pneumoniae* culture was found to produce a well-defined, 2–3 day febrile illness, followed in most instances by spontaneous recovery. This illness therefore permitted sequential

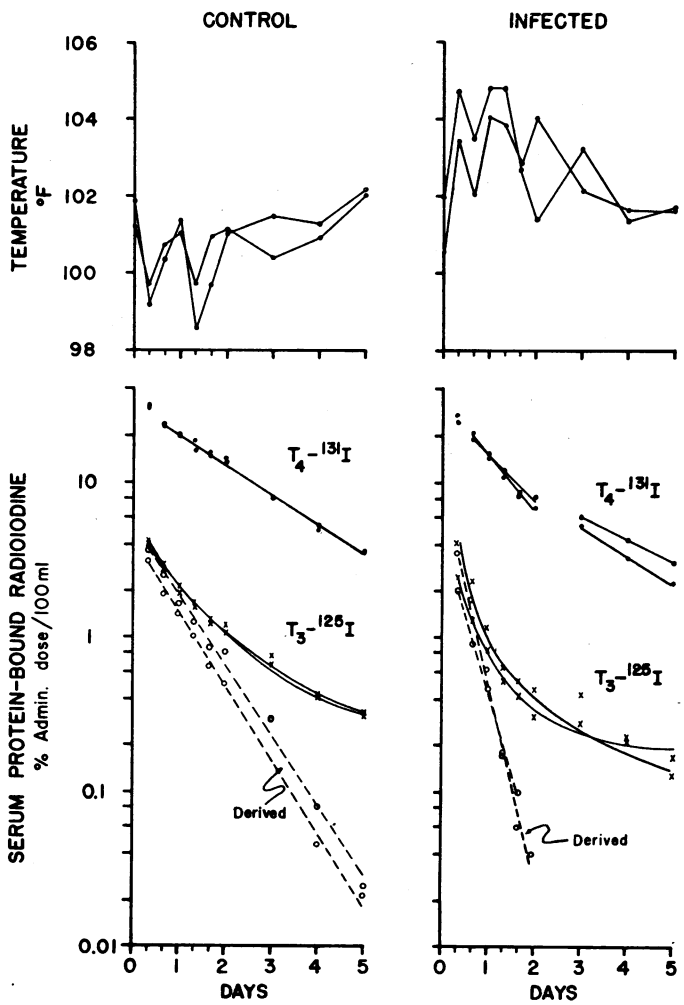

FIGURE 3 The effects of acute infection with *Diplococcus pneumoniae* on the disappearance from serum of ^{131}I-labeled L-thyroxine (T_4-^{131}I) and of ^{125}I-labeled 3,3',5-triiodo-L-thyronine (T_3-^{125}I) administered concurrently. The time of inoculation with the culture was at 0 time and was followed immediately by injection of the labeled hormones. In the two control monkeys, the disappearance curves for T_4-^{131}I were virtually superimposeable.

TABLE IV

The Effect of Acute Infection with Diplococcus pneumoniae In Vivo on the Deiodination of ^{131}I-Labeled L-Thyroxine (T_4-^{131}I) by Leukocytes In Vitro

Day*	Monkey inoculated with heat-killed culture					Monkey inoculated with virulent culture				
	Leukocytes in whole blood			Leukocytes in incubate	T_4-^{131}I deiodination	Leukocytes in whole blood			Leukocytes in incubate	T_4-^{131}I deiodination
	Total	PMN	Bands			Total	PMN	Bands		
	number/μl	%	%	*number/μl*	%	*number/μl*	%	%	*number/μl*	%
−1	8100	17		3500	4.3 (4.02, 4.56)‡	14,750	8		2700	1.6 (1.59, 1.66)
0	8250	8		2750	4.0 (3.85, 4.23)	13,200	13		2700	4.5 (4.22, 4.70)
0.25	14,000	41		2500	3.5 (3.49, 3.57)	15,500	51		2050	11.8 (11.56, 12.01)
1	15,200	72	1	3400	4.2 (4.15, 4.26)	15,100	54	1	3000	3.7 (3.62, 3.79)
2	10,100	26		2800	7.9 (7.60, 8.24)	13,900	57	11	3050	3.0 (2.82, 3.26)
3	9700	25	1	2400	5.0 (4.90, 5.07)	11,600	48	8	2900	2.8 (2.71, 2.89)
6	7900	33	1	2660	1.8 (1.53, 2.15)	26,100	55	4	3140	3.0 (2.09, 3.99)

* The time of inoculation is designated as day 0 and immediately followed the collection of blood for the measurements shown.
‡ The values in parentheses are the values in duplicate flasks.

observations to be made during progression from the healthy state through acute infection into convalescence.

The earliest detectable alteration following inoculation with the virulent culture was accelerated clearance of both T_4 and T_3 from their respective peripheral pools. A similar alteration has previously been observed in man during the acute phase of pneumococcal pneumonia (3). This alteration was often evident by 8 hr after inoculation, as judged from the lower values for the concentration of labeled hormone in serum at this time relative to the control values. A decrease in extracellular binding of hormone did not appear to be responsible for the accelerated hormonal clearance for two reasons. First, no alteration in the distribution of labeled T_4 among the binding proteins was observed in serum obtained during the acute febrile period. Second, the fractional rates of disappearance of both T_4 and T_3 from serum were increased during this period. This is contrary to what would be expected were a decrease in extracellular binding responsible, since alterations in extracellular binding induce alterations in the fractional rates of T_4 and T_3 disappearance that are the converse of one another (8, 9). Rather, the data are more in keeping with enhanced cellular uptake and metabolism of both hormones during the acute febrile period. The role of fever in the pathogenesis of this alteration cannot be assessed in our study owing to the relatively small number of animals employed. However, in the study of acute pneumococcal pneumonia in man cited above (3), no correlation appeared to exist between the magnitude of the febrile response and the acceleration of hormonal disappearance.

During the convalescent period, the increased volume of T_4 distribution persisted, but a decreased fractional rate of disappearance was observed. The latter phenomenon has also previously been observed during recovery from acute pneumococcal pneumonia in man (3). The decreased fractional rate of disappearance might have been ascribed either to more rapid thyroidal recycling of labeled iodide liberated from more rapid peripheral degradation of hormone or to more rapid formation of the iodoprotein product of T_4 metabolism, or to both. Both these explanations would be consistent with an increased flux of hormone to the cells during the preceding acute febrile period. However, the T_4-^{131}I that was administered as a fresh injection during the convalescent period behaved in a similar fashion. Not only was its volume of distribution greater and its rate of disappearance from serum less than the control values, but in no instance was a progressive slowing of the disappearance curve observed. Consequently, neither increased thyroidal recycling of liberated iodide nor increased iodoprotein accumulation could be implicated as a major factor in the slowing that was observed.

Another factor that might have been implicated in the slowing of the fractional rate of hormonal disappearance was an increase in extracellular binding. On day 6 during the convalescent period, a slightly greater proportion of T_4 was associated with TBG in serum enriched with approximately 70 μg of T_4 per 100 ml. Although the degree of saturation of TBG was not assessed owing to the small supply of serum, this concentration of added T_4 should approach that required to measure binding capacity if the binding capacity of TBG in rhesus serum is similar to that in man. Consequently, the increased proportion of T_4 associated with TBG reflected in all likelihood an increased binding capacity of the protein. Nevertheless, it is unlikely that the increase in extracellular binding was the sole mechanism responsible for the alterations in the kinetics of hormonal disappearance during the convalescent period because an abrupt decrease in the volume of distribution did not occur; in fact, the volume of distribution was consistently greater than that observed during the control period. Rather, the data suggest that increased cellular uptake of T_4 persists into the convalescent period (and this would be in accord with the persistent increase in the volume of T_4 distribution that was observed), but that its access to sites of rapid metabolism is retarded by the T_4 accumulated at an increased rate during the preceding acute febrile period.

The behavior of the endogenously synthesized, labeled hormone is also of some interest. During the acute febrile period, its concentration in the sera of the four surviving monkeys either remained essentially unchanged or increased in the face of an increased rate of hormonal clearance, indicating that the rate of hormonal secretion must have increased during this period. Although a direct comparison between the endogenously labeled hormone and the endogenous stable T_4 is not possible owing to the fact that the latter represents the value for several samples of serum pooled during each period, the failure of serum T_4 to decline in the face of accelerated hormonal clearance also supports increased hormonal secretion as occurring during the acute febrile period. The mechanism by which this increase was evoked is not known. However, it is tempting to speculate that it might have been secondary to the accelerated hormonal clearance. In one of the two nonsurvivors, the concentration of endogenously labeled hormone was depressed, reflecting the greatly accelerated hormonal clearance, whereas in the other the concentration in serum tended to fluctuate widely, suggesting possibly a transient burst in hormonal secretion.

Our data, as well as those obtained previously in man during acute pneumococcal pneumonia (3), indicate that during the acute febrile period the flux of hormone to the cells is increased. Accordingly, the question to which we next addressed ourselves concerned the role subserved by this increased cellular availability of hormone. We therefore directed our attention to the peripheral blood leukocytes. This was prompted both by their ready accessibility and by the observation of Klebanoff (10) that the phagocytosis of bacteria is followed by their iodination and that this may represent a microbicidal mechanism in the leukocyte. Many peripheral

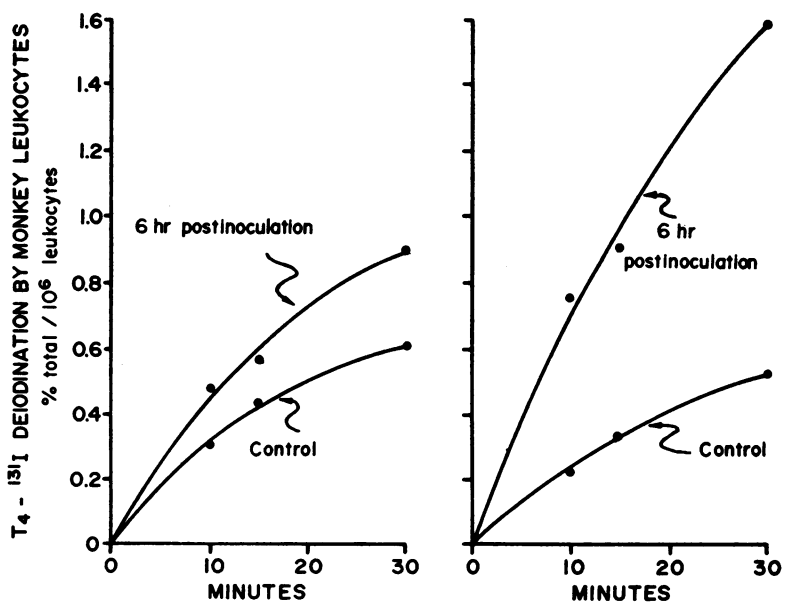

FIGURE 4 Comparison of the effects of inoculation with the heat-killed (left graph) and virulent (right graph) *Diplococcus pneumoniae* cultures on the rate of T_4-^{131}I deiodination by leukocytes in vitro.

tissues possess a T_4-dehalogenase, and one of the products of this dehalogenation reaction is iodine in a relatively oxidized state (11) that should be capable of iodinating bacteria. In the present study, leukocytes obtained from monkeys 6 hr after inoculation with the virulent culture displayed enhanced T_4-deiodinative activity, whether assessed by early rate measurements or by plateau measurements. This did not appear to be related to the alteration in leukocyte population because a similar alteration followed inoculation with the heat-killed culture, and this was accompanied by little or no change in deiodinative activity. During this early period after inoculation, the bacteria are undergoing multiplication and are being rapidly phagocytosed. Thus, our observation could be interpreted as providing a source of readily available iodine in a relatively oxidized state for bacterial iodination though we have no evidence that bears directly on this point. On the other hand, since the T_4-dehalogenase may be a peroxidase (12) and since phagocytosis is accompanied by increased peroxidatic activity and hydrogen peroxide generation (13–15), it is entirely possible that the enhanced deiodinative activity of leukocytes was merely an accompanying phenomenon unrelated to any microbicidal action.

The observed alterations in over-all hormonal economy, however, cannot be ascribed to the enhanced deiodinative activity of the peripheral blood leukocytes for this was short-lived. Consequently, enhanced cellular uptake of hormone must have occurred in other sites. What these sites are and what role the hormone therein subserves during an acute bacterial infection remains to be determined. Finally, the similarity between the present data and that obtained previously in man during acute pneumococcal pneumonia (3) suggests that the rhesus monkey is a suitable animal model for studying the influence of infection-related stress on thyroid hormone economy.

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