MARKED SUPPRESSION OF THYROID FUNCTION IN RATS WITH GRAM-NEGATIVE SEPTICEMIA

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Gram-negative septicemia was induced in rats by two daily injections of fecal mixture into the thigh, after which the thyroid function was markedly suppressed for 2 days. Iodine metabolism was studied by organ radioassay and by imaging with a multiwire proportional chamber (MWPC) at various time intervals after intravenous injection of ^{125}I. Plasma T_3, T_4, and TSH, measured by radioimmunoassays, were suppressed, as were the T_3-resin uptakes. Fractional blood supply to the thyroid glands of the infected rats, studied by the ^{81}Rb uptake method, was also found to be markedly reduced. Sections of the thyroid glands showed little structural change during the period of marked thyroid suppression. There was no biochemical evidence of renal failure in the septicemic rats.

In spite of the voluminous literature on thyroid function, only a few reports on thyroid function during acute infection have appeared. Cole and Womack (*1*) reported that iodine concentration in the thyroid glands of infected dogs was much lower than in healthy dogs. Sternberg et al (*2*) showed that the thyroid uptake of ^{131}I in mice was suppressed during infection with coccidioidomycosis and sporotrichosis. Reichlin and Glaser (*3*) found that thyroid function, measured by the discharge rate of ^{131}I, was suppressed during experimental streptococcal pneumonia in the rat. More recently, Shambaugh and Beisel (*4*) infected rats subcutaneously with type-specific pneumococci. They found decreased thyroid uptake of ^{131}I, depressed thyroid responsiveness to TSH, decreased T_4 binding in blood, and an associated shorter half-time in the circulation.

Although studies in humans are few in number, reports on infectious hepatitis (*5*), leprosy (*6*), schistosomiasis (*7*), and Pasteurella tularensis (*8*) are available. The results from these studies are variable and difficult to interpret because of differences in the severity of infection and in the therapeutic treatment. Although the thyroid ^{131}I uptake was suppressed in infectious hepatitis with severe damage to the liver (*5*), thyroid function was unchanged in leprosy (*6*).

The current study confirmed the suppression of thyroid function in rats with gram-negative infection. Imaging of the whole-body distribution of ^{125}I was facilitated by a new camera system, the multiwire proportional chamber of the Lawrence Radiation Laboratory. Accurate measurement of plasma TSH was also made possible with the recent development of radioimmunoassay procedures. This is also the first report on local thyroid blood supply during septicemia.

MATERIALS AND METHODS

Induction of infection. All rats used in our study were male Sprague–Dawley rats weighing 200–250 gm. Control and infected rats used within the same experiment were born on the same day. Because of its easy availability in the laboratory, fecal material from the rats was used as the infective agent. Approximately 5 gm of rat feces were softened in normal saline, then wrapped in a double layer of cheese cloth, and submerged in 30–60 ml of 5% dextrose. By squeezing the contents within the cheese cloth repeatedly with forceps, a turbid mixture of fecal material was obtained.

Under ether anesthesia, the rats were given 1 ml of fecal mixture intramuscularly in the right thigh. On the following day, these rats showed moderate edema and cellulitis of the right thigh, but they were generally quite alert. These rats were used for the 3-hr ^{125}I uptake and organ distribution studies; they

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served as a model for local and relatively mild infection. In order to produce septicemia, the rats were given a second injection of 1.5 ml of the fecal mixture in the swollen right thigh 1 day after the first injection. On the following day, the rats were clinically quite sick, being dull and slow in reaction, with their hair ruffled and coarse and their right lower limbs and occasionally their lower abdominal wall swollen from the spreading cellulitis. Eleven of 142 rats injected this way died overnight, but those that survived the first day gradually recovered in spite of obvious weight loss.

In order to ensure that the suppression of thyroid function was not due to anesthesia or to the injection process, the 3-hr ^{125}I uptake study included 12 control rats that received injections of 5% dextrose under ether anesthesia. These rats showed no observable effects from the injections.

Blood culture. Ten rats infected by two daily fecal injections and six normal rats were used for blood culture. Using aseptic techniques, 5 ml of blood from each rat was drawn into a heparinized syringe and then added to blood culture bottles from Hyland Laboratories (Costa Mesa, Calif.) or from Roche Diagnostics (Nutley, N.J.). Biochemical tests for identification of organisms were carried out in Enterotube from Roche Diagnostics. Seven of ten infected rats showed positive growth of gram-negative organisms. These were identified as Escherichia coli in five rats and Proteus mirabilis in the other two. All six control rats showed negative blood cultures.

Imaging study with the multiwire proportional chamber (MWPC). This relatively new instrument in nuclear medicine (*9*) is ideal for imaging the organ distribution of ^{125}I in rats. It has a better intrinsic resolution than the Anger camera and its counting efficiency for low-energy gamma emitters such as ^{125}I is good. The MWPC was filled with a gas mixture of 90% Xe and 10% CO_2 at atmospheric pressure; it had a large active area of 20 × 20 in. for imaging. A parallel-hole collimator with a resolution of approximately 2 mm at the surface was used for imaging.

Rats for imaging were given 200–300 μCi of ^{125}I as carrier-free sodium iodide solution (Mallinckrodt/Nuclear, St. Louis, Mo.) by injection into the tail veins. They were killed by ether at 15 min, 30 min, 1 hr, 3 hr, and 24 hr after injection, and anterior views were obtained by the MWPC. The whole-body distribution of ^{125}I was recorded on Polaroid films, 50,000 counts being obtained from each rat. The high voltage for the central-plane wires of the MWPC was set at 3,000 volts, with a 40% window for ^{125}I.

Plasma T_3, T_4, TSH, and T_3-resin uptake. Plasma T_3 and T_4 were determined by radioimmunoassay procedures using specific antiserum for T_3 and competitive binding for T_4. Measurements of T_3-resin uptake were performed using the Thyopac (Amersham/Searle, Arlington Heights, Ill.) and the results were given as the percent of binding of the human normal mean value. Rat TSH for radioimmunoassay and for organ distribution studies had a biologic potency of 35 I.U./mg by the McKenzie Mouse Assay. Radioiodination of rat TSH was based on the chloramine-T method of Greenwood, Hunter, and Glover (*10*) as described in detail by Garcia et al (*11*). The specific activity of ^{125}I-TSH obtained this way was approximately 200 μCi/μg. Radioimmunoassay was performed using a double-antibody technique (*11*).

Iodine-125-TSH organ distribution study. Six normal and six septicemic rats were given 20 μCi of ^{125}I-TSH that had been purified by Sephadex G-50 column. They were killed at 30 and 60 min after injection for organ counting. The thyroid glands were removed from the trachea and counted against a 0.1% standard in a well counter.

Rubidium-81 perfusion study. Fractional blood supply to the thyroid gland was studied using the uptake method of Sapirstein (*12*), who showed that the content of ^{42}K or ^{86}Rb uptake in the organ reflected its fractional blood supply. In our study, 50 μCi of ^{81}Rb-rubidium chloride (Medi-Physics, Emeryville, Calif.) in 0.5 ml normal saline was injected into the tail or femoral vein of rats sedated with 10 mg intraperitoneal pentobarbital. The rats were killed by removing the hearts 30 sec after injection. The thyroid glands were removed from the trachea and counted against a 0.1% standard. They were then weighed by a sensitive balance. The ^{81}Rb uptake was expressed as percent of injected dose per organ and per gram of thyroid tissue.

Plasma creatinine. The renal function of 20 normal and 17 septicemic rats was studied by measuring the plasma creatinine, using Jaffe's reaction between alkaline picrate and creatinine.

Pathologic study. The gross appearance of the thyroid gland in normal and septicemic rats was studied with a binocular dissecting microscope. The thyroid glands were fixed in Bouin's solution and stained by hematoxylin and eosin for microscopic study.

RESULTS

The thyroid function in the septicemic rats was markedly suppressed as measured by ^{125}I, T_4, and T_3-resin uptake. Figure 1 shows the MWPC picture of normal and septicemic rats after injection of ^{125}I. Whereas the concentrations of ^{125}I at 30 min and 1 hr after injection are sufficient to show the thyroid gland in the normal rat, it cannot be seen in the septicemic rat. Figure 2 shows that the ^{125}I thyroid uptake of the septicemic rats is lower than the normal controls at all time intervals after injection. The de-

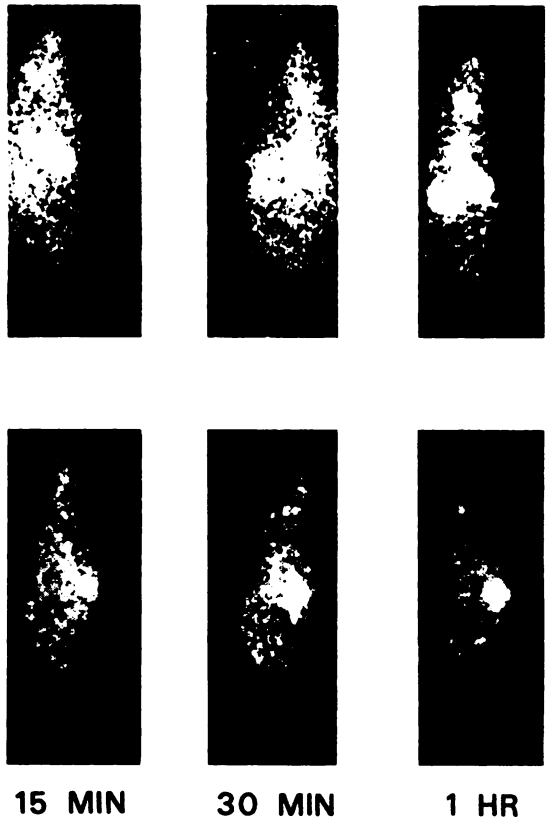

FIG. 1. MWPC pictures of thyroids of normal (top) and septicemic (bottom) rats, showing poor concentration of ^{125}I in septicemic rats.

gree of thyroid suppression is also related to the severity of infection. For example, the 3-hr ^{125}I uptakes for rats given one, two, or three injections of fecal mixture are 2.52 ± 1.01%, 1.17 ± 0.43%, 0.91 ± 0.16%, respectively, compared with 3.86 ± 1.32% for normal rats.

The Plasma T_3, T_4, T_3-resin uptake and TSH are all suppressed in the septicemic group. Table 1 summarizes the mean values and their standard deviation.

With the exception of the thyroid gland, the ^{125}I-TSH distribution study showed essentially similar results for the normal and septicemic rats. At 30 min after injection the percent uptake is 0.065 ± 0.011% for the normal rats and 0.028 ± 0.007% for the infected rats.

The thyroid ^{81}Rb uptake study showed that the fractional blood supply to the thyroid gland was suppressed in septicemic rats. Expressed as percent of injected dose per gram of thyroid tissue, the uptake is 3.37 ± 1.17% for normal rats and 1.85 ± 0.85% for septicemic rats, with no overlap between the two groups.

Consistent with the reduced blood flow, the thyroids in the septicemic rats look paler than the normal controls under tenfold magnification. Histologic study, however, does not reveal any significant change in the thyroid in the first 3 days after induction of septicemia. There was no difference between the plasma creatinine levels for the two groups.

TABLE 1. T_3, T_4, AND T_3-RESIN UPTAKE AND TSH IN NORMAL AND SEPTICEMIC RATS

	Normal rats	Septicemic rats
T_3 (ng/100 ml)	22.86 ± 7.73	Less than 12.0
T_4 (μg/100 ml)	4.13 ± 1.08	1.23 ± 0.43
T_3-resin uptake (% human normal)	57.57 ± 3.21	42.71 ± 1.38
TSH (10^{-6} I.U./ml)	118.05 ± 45.82	69.47 ± 34.24

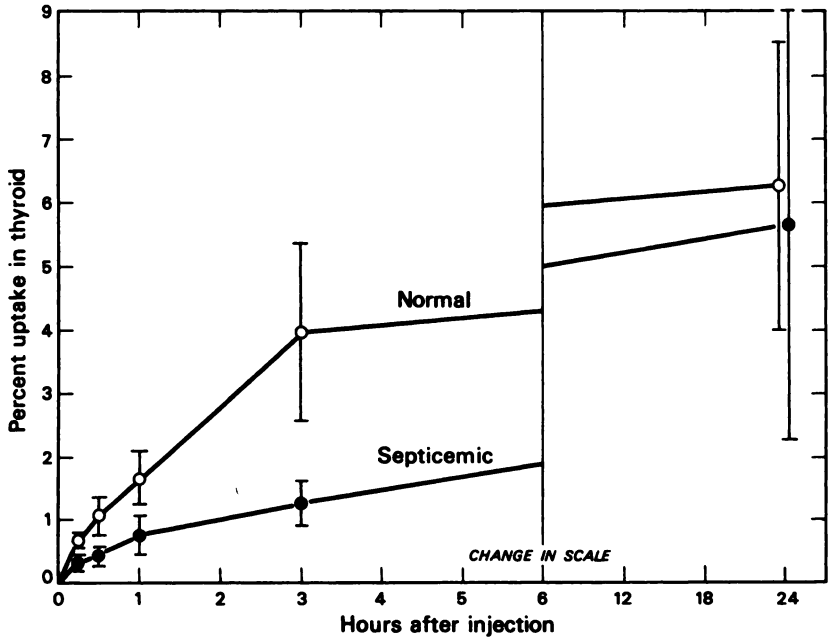

FIG. 2. Thyroid ^{125}I uptake in normal and septicemic rats.

DISCUSSION

By all parameters studied, the thyroid function of rats was markedly suppressed during gram-negative septicemia induced by fecal injection. In spite of the functional changes, no histologic or weight difference is observed in the first 3 days of infection. The exact mechanism by which thyroid function is suppressed during infection has not previously been elucidated. The adrenal gland is not responsible for the change because thyroid inhibition occurs even in adrenalectomized animals on constant cortisone intake (*3*). From the observations of Brown-Grant, Harris, and Reichlin (*13*), it has been inferred that severe physical stress may reduce the responsiveness of the thyroid gland to TSH and depress pituitary output as well. Attempts have been made to solve the problem by measuring the plasma TSH level but the low sensitivity of bioassay methods does not permit accurate measurements of plasma TSH below the normal range. Using a reliable and sensitive double-antibody radioimmunoassay method, we have shown that the plasma TSH was significantly lower in the septicemic rats ($p < 0.001$).

In addition to the effect of TSH, Badrick et al (*14*) suggested the participation of local metabolic or vascular factors in the suppression of thyroid function since severe physical stress reduced thyroid activity even in hypophysectomized animals. Our ^{81}Rb data also suggest that the reduction of blood supply to the thyroid gland of septicemic rats may be involved in its functional inhibition. A combination of decreased TSH production and reduced fractional blood supply to the thyroid gland is the probable cause for the suppression.

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