the thromboplastic suspension and identified as *B. subtilis*. Inoculation of thromboplastic suspension with a physiologically active form of *B. subtilis* drastically reduced the interval before the abrupt prolongation of clotting time. This also could be prevented (up to 7 hours at 37.5°C at least) with 0.25% phenol. The fact that *B. subtilis* can alter the apparent thromboplastic potency of tissue suspensions seems to have practical value as well as theoretical promise. From the practical aspect, the utilization of thromboplastic suspension can be extended by simple addition of phenol in cases where the potency loss is due to bacterial action. From the theoretical aspect, it would appear that the △ suspensions have an altered activator-inhibitor content. Such suspensions are amenable to investigation leading to possible concentration, or separation of activator or inhibitor. It is apparent from these preliminary observations that the nature of the changes of the incubated thromboplastic suspensions are yet to be explained. It is clear, however, that the marked changes induced by this bacterium and controlled by simple conditions offer new methods of investigating the content and nature of thromboplastic materials without resorting to drastic physicochemical treatment.

Summary. Data have been presented which show that a bacterial contaminant identified as *B. subtilis* caused a sudden deterioration in potency of brain and lung thromboplastic suspensions, which could be prevented by various agents. Some aspects of the functioning of the altered thromboplastic suspensions are presented and their significance discussed.

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Effects of Thyroidectomy and of Thiouracil on Adrenal Weight and Ascorbic Acid. (18322)

HENRY H. FREEDMAN, AND ALBERT S. GORDON.

From the Department of Biology, Washington Square College of Arts and Science, New York University.

It has been demonstrated that thyroid substances induce an enlargement of the adrenal glands(1,2), whereas thyroidectomy, or the administration of goitrogenic substances, results in a decrease in adrenal size(2-4). These alterations in adrenal size are restricted to the cortex of the gland. Long(5) and Sayers and Sayers(6) have reviewed the recent wide use of adrenal ascorbic acid content as an index of adrenocortical activity. The changes in adrenal ascorbic acid following adrenal stimulation or depression are also confined to the cortex. The effects of thyroid material on adrenal size and ascorbic acid content have already been reported by Sure and Theis(7) and by Wallach and Reineke(8). The present investigation was undertaken to determine the effects of hypothyroidism on the adrenal weight and adrenal ascorbic acid concentration of the male rat. Since it has been shown by Gordon, Goldsmith, and Charipper(9) that thyroidectomy and thiouracil exert somewhat

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TABLE I. Effects of Hypothyroidism on Adrenal Weight and Ascorbic Acid Concentration After 1, 2, and 4 Weeks of Treatment.

Wk	Group	Body wt,* g	Gain or loss,† g	Adrenal wt, mg	Ascorbic acid μg/mg adrenal	Thyroid wt, mg
1	1	157 ± 3	+24 ± 3	18.5 ± 2.2	5.67 ± .15	26.8 ± .9
	2	159 ± 7	+27 ± 1	22.3 ± 1.3	5.20 ± .33	15.1 ± .7‡
	3	167 ± 8	+31 ± 4	23.9 ± 2.1	5.59 ± .10	16.8 ± .6
	4	183 ± 9	— 4 ± 3	23.6 ± 1.7	5.71 ± .17	—
	5	190 ± 17	— 3 ± 8	27.3 ± 1.2	5.80 ± .20	—
2	1	133 ± 4	+42 ± 1	17.4 ± 0.7	4.70 ± .13	37.5 ± 1.3
	2	135 ± 4	+44 ± 1	21.0 ± 0.6	4.76 ± .35	14.9 ± .7
	3	133 ± 9	+46 ± 4	24.1 ± 1.4	5.57 ± .19	15.5 ± 1.1
4	1	153 ± 8	+38 ± 3	15.8 ± 0.8	4.83 ± .14	73.7 ± 2.5
	2	199 ± 6	+61 ± 2	24.3 ± 1.3	4.33 ± .14	21.2 ± .7
	3	184 ± 7	+44 ± 3	27.2 ± 1.7	4.31 ± .22	17.0 ± 1.4
	4	253 ± 16	+33 ± 5	29.9 ± 2.2	3.67 ± .25	—
	5	212 ± 8	+17 ± 4	32.8 ± 1.8	4.74 ± .17	—
Controls§		133 ± 5	—	22.4 ± 0.8	4.63 ± .07‖	16.1 ± .7¶
		158 ± 7	—	24.0 ± 1.4		
		189 ± 4	—	27.1 ± 0.9		
		227 ± 3	—	28.9 ± 1.5		

All values are means ± standard error. Group 1, thiouracil; 2, thiouracil + thyroxin; 3, thyroxin; 4, thyroidectomy; 5, thyroidectomy + thyroxin. Five rats per group in each period.
* At sacrifice. † Final minus initial body weight. ‡ 4 animals. § 6 animals per group. ‖ Value for all controls(25). ¶ Value for 12 animals.

different effects on metabolism, both methods of inducing hypothyroidism were employed. In addition, an estimated physiological dose (8) of thyroxin was administered daily to animals of both hypothyroid groups to determine whether such thyroidal replacement would prevent the adrenal changes associated with the experimental treatment.

Materials and methods. Sixty-five young male rats of a modified Long-Evans strain were separated into 5 experimental groups. The animals in Group 1 received thiouracil mixed thoroughly in their powdered complete ration in a concentration of 0.2%. Group 2 received the same thiouracil-supplemented diet plus daily subcutaneous injections of 5 γ of d,l-thyroxin per 100 g body weight in 0.1 cc saline. Group 3 received only the thyroxin. The animals in Group 4 were surgically thyroidectomized under ether anesthesia. Group 5 was thyroidectomized and also received 5 γ of thyroxin per 100 g body weight per day beginning with the first day of operation. Groups 1 through 3 contained 15 animals each, which were sacrificed in groups of 5 after 1, 2, and 4 weeks of treatment. Groups 4 and 5 contained 10 animals each, which were sacrificed in groups of 5 at 1 and 4 weeks after the start of treatment. One control group of 3 animals received subcutaneous injections of 0.1 cc of saline per day for one week, and another group of 3 animals were sham-operated and sacrificed one week later. A group of 25 animals was used for determination of normal adrenal weight and ascorbic acid, and normal thyroid weight was ascertained in 12 of these animals.

All animals were sacrificed by exsanguination. The adrenals were dissected out, cleanly trimmed of adhering fat, and weighed to the nearest tenth of a milligram on a torsion balance. The thyroids, in Groups 1 through 3, were also removed and weighed. After weighing, the adrenals of each animal were extracted with portions of a 6% solution of trichloroacetic acid, and adrenal ascorbic acid was then determined by the method of Roe and Kuether(10) with the dinitrophenylhydrazine reagent prepared by the method of Lowry, Lopez, and Bessey(11).

Results. The data obtained after 1, 2, and 4 weeks of treatment are shown in Table I, with values for normal animals of the same

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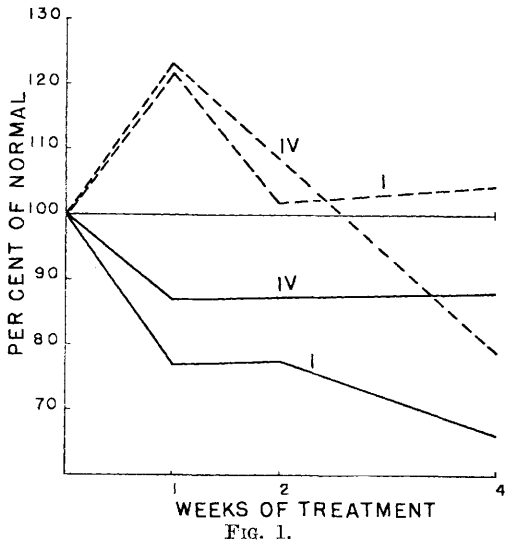

FIG. 1.

Adrenal ascorbic acid concentration (broken line), and adrenal weight (solid line), as % of normal after 1, 2, and 4 weeks of treatment. I, thiouracil group; IV, thyroidectomized group.

body weight.

The adrenal ascorbic acid concentrations and the thyroid weights of the controls showed no significant changes over the body weight range used and, therefore, the values for all control animals were pooled. On the other hand, the adrenal weights varied significantly with body weight in the normal animals, so that adrenal weights in each of the experimental groups are compared to those of normals of the same body weight range.

It can be seen from Fig. 1 that the adrenal atrophy was greater in the thiouracil-treated than in the thyroidectomized group; this same result was found by LeBlond and Hoff(3) and by Deane and Greep(2) with approximately the same concentration of thiouracil. Three sham-operated animals (not shown in the table) were found to have adrenal weight values 8% higher than normal after 1 week.

The data of Fig. 1 indicate that in Group 1 the increase in ascorbic acid concentration at the end of the first week was equivalent in magnitude to the decrease in adrenal weight; at 2 and 4 weeks the concentrations did not differ significantly from normal. In Group 4, however, the increase in ascorbic acid concentration at 1 week and the fall below normal at 4 weeks exceeded in magnitude the adrenal weight changes observed at the same times.

Fig. 2 shows that the daily dose of thyroxin administered to thiouracil-treated animals overcame almost completely the effects of the goitrogen. At 4 weeks, however, there was still a slight thyroid enlargement. The decreased food intake of thyroidectomized or thiouracil-treated animals has been reported (9,12). It was noted in this experiment that the animals receiving thyroxin in addition to thiouracil had a greater food consumption than those receiving only the goitrogen. Thus the animals in Group 2 consumed a greater absolute amount of the goitrogenic compound per day than did the animals in Group 1.

In Group 5 (Fig. 2) the adrenal weights did not differ from normal at any of the experimental periods, and the ascorbic acid concentration did not fall below normal at 4 weeks as in Group 4.

The level of thyroidal stimulation employed in Group 3 (Fig. 2) gave results approximating those obtained by Wallach and Reineke (8) who used a greater dosage. The ascorbic acid concentration in the adrenals of 3 animals receiving 0.1 cc of saline per day for 1 week

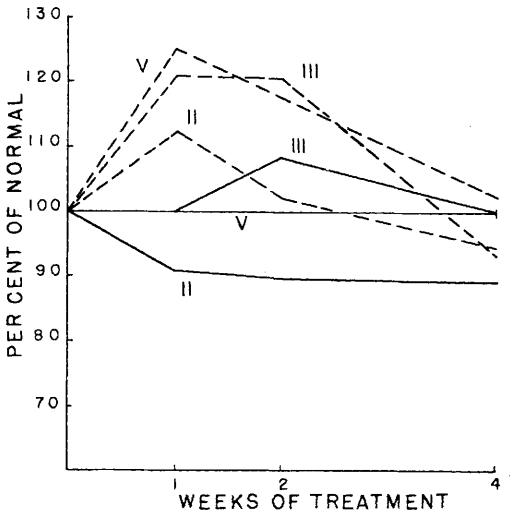

FIG. 2.

Adrenal ascorbic acid concentration (broken line), and adrenal weight (solid line), as % of normal after 1, 2, and 4 weeks of treatment. II, thiouracil + thyroxin group; III, thyroxin group; V, thyroidectomized + thyroxin group.

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(not shown in the Table) was not found to be significantly different from normal.

Discussion. The initial high ascorbic acid concentration seen after 1 week of treatment in the thiouracil-fed group, equivalent in magnitude to the atrophy of the adrenals, may reflect an increased storage by the adrenal cortex which, at this time, retains the normal total content of the vitamin. The normal ascorbic acid values noted in the later periods, in the atrophied glands, are interpreted as indicating an adjusted rate of synthesis, or mobilization, of the vitamin, with each unit mass of tissue secreting at a normal rate but the total adrenocortical output much reduced.

In the thyroidectomized rats and the thyroidectomized animals given thyroxin the initial increase, by the first week, in adrenal ascorbic acid probably represents a later phase of the response to the stress of the operation. The importance of the role of stress at the first week following the operation is suggested by the adrenal hypertrophy of the sham-operated animals, and by a previous observation (unpublished) that there is a sharp drop in adrenal ascorbic acid within 48 hours after the removal of the thyroids. The final decrease (at 4 weeks) in adrenal ascorbic acid concentration in the thyroidectomized animals exceeds the atrophy of the adrenals, and is believed to demonstrate the eventually greater effectiveness of thyroidectomy than of thiouracil in repression of cortical function. These results indicate a decreased rate of synthesis, or mobilization, of the vitamin and a more greatly reduced adrenocortical output, probably adequate, however, to handle the lowered metabolic needs of this type of animal(6).

The data obtained with thyroidal replacement in both hypothyroid groups support the view that the observed adrenal changes are due actually to changes in the level of thyroidal function. Experiments are required to determine whether the adrenal changes in hypothyroidism result from a decrease in the output of adrenocorticotrophic hormone (perhaps because of the accelerated production and release of thyrotrophic hormone by the pituitary) or whether the adrenal also responds directly to the lowered levels of thyroid hormone in the blood. In prolonged stress Pinchot, *et al.*(13) have questioned the reliability of ascorbic acid alone as an index of cortical activity. The results reported here demonstrate that the wide use of adrenal ascorbic acid concentration as an index of adrenocortical function is valid only if the changes are interpreted in terms of alteration in adrenal size.

Summary. The administration of thiouracil to the male rat results in a pronounced atrophy of the adrenal glands and an initial increase in ascorbic acid concentration in the adrenals followed by a return to normal values. Surgical thyroidectomy produces a less pronounced adrenal atrophy but a greater adrenal inhibition as revealed by a final decrease in adrenal ascorbic acid concentration. The effects of both types of induced hypothyroidism on the adrenals may be prevented by the administration of thyroxin. It is concluded that adrenal ascorbic acid concentrations are reliable indices of adrenocortical activity only if interpreted in terms of change in adrenal size.

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