Beta-Glucuronidase in Leukocytes, Plasma, and Urine of Patients with Nephrosis Undergoing Treatment with ACTH.* (18910)

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(Introduced by E. A. Zeller.)

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B-glucuronidase has been associated with the metabolism of glucuronic acid, of the estrogens, and of alkaloid poisons such as borneol. It has also been shown that this enzyme's activity is increased in malignant tissue and that cortical steroids are excreted for the most part as glucuronides. Fishman(1) showed that activity of mouse liver was increased following administration of borneol and menthol which are conjugated by the animal into the glucuronide form. Later he showed that mouse uterus activity is increased by administration of estrogens thus suggesting the possibility of an active conjugated form of the estrogen(2). Odell and Fishman(3) have shown that the endometrium has cyclic changes paralleling that of the menstrual cycle. Blood and urine glucuronidase have been found to be increasingly elevated during pregnancy(4). Fishman noted an elevation of B-glucuronidase activity in carcinomas of various organs(5) and recently there has been an attempt made to develop a "limited selective test for cervical (cancer) suspects"(6) by determining the B-glucuronidase activity of vaginal fluid. It has been also shown that the corticosteroids in human urine are for the most part present as glucuronic acid conjugates(7). Although these many facts about the enzyme have been ascertained, its physiological significance is not yet absolutely clear since the precise mode of action of the enzyme *in vivo* is still somewhat obscure.

We have employed in our laboratory for a considerable time the glucuronidase hydrolysis method for the measurement of the corticosteroids excreted in the urine of patients before, during, and after administration of adrenocorticotropin (Armour) in order to assay the degree of cortical activity elicited by the ACTH and to compare this with clinical response. In addition, from time to time, we have determined the free corticosteroids present in the urine. From this we have seen that favorable clinical response does not absolutely follow an increased cortical output (although in the cases of striking improvement a potent activation was achieved) and have also noted that the ratio between the free and total corticosteroids does not remain constant during ACTH therapy. Since the cortical hormones are demonstrably associated with glucuronic acid and since glucuronidase, capable of either hydrolysis or conjugation of the hormones, might be responsible for the difference in clinical response by reason of its supplying an abundance of the active form, it was decided to investigate the B-glucuronidase activity of blood and urine.

Methods. Glucuronidase assay: The beta-glucuronidase was assayed by the method of Fishman, Springer, and Brunetti(8). The method was found to give very reliable duplicates. The transmittance readings agreed within 1%. Control tubes were prepared containing (1) substrate and buffer (2) sample without substrate. The results were calculated as averages of the duplicates. According to the recommendations of Fishman,

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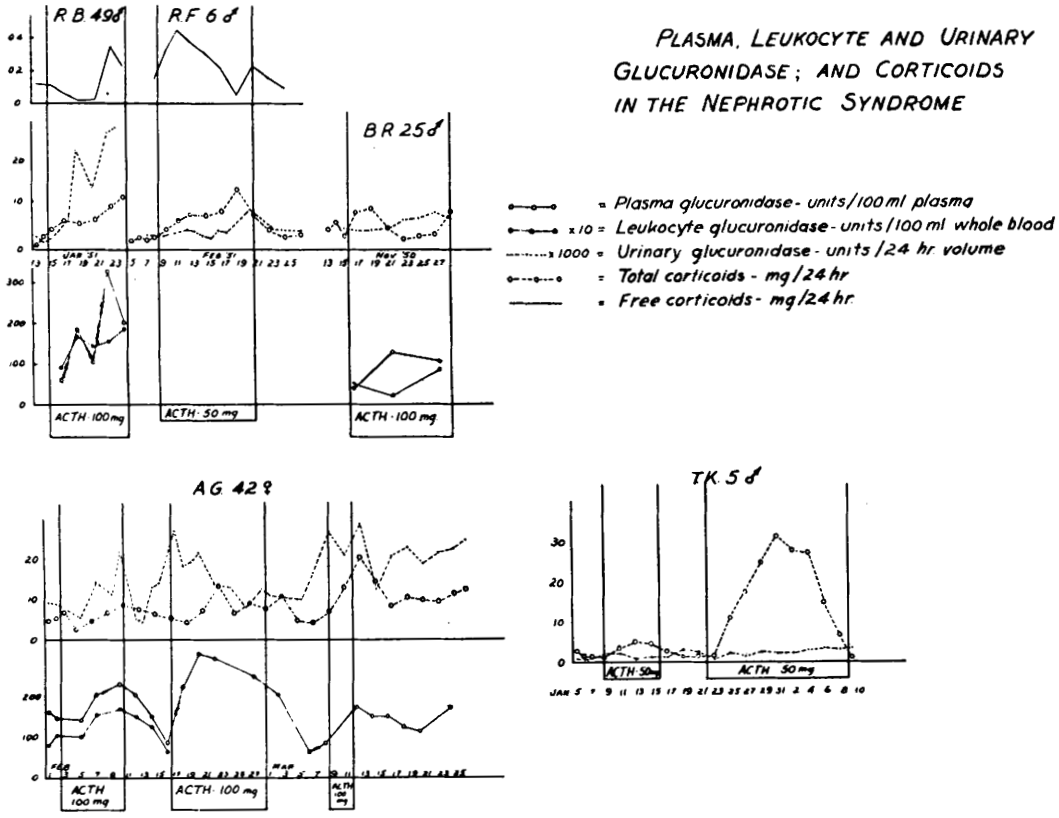

FIG. 1.

the urine samples were not subjected to dialysis. One enzyme unit refers to the liberation of 1 γ per hour of phenolphthalein. 0.1 cc of urine was used with the recommended buffers and substrate and incubated at 37.5°C for 10-20 hours.

Free Corticosteroid Assay: Free urinary corticosteroids were determined by analyzing the fresh, 24 hour urine without acidification and without enzymatic hydrolysis.

Total Corticosteroid Assay: Total urinary corticosteroids were obtained by hydrolyzing 25 ml aliquots of 24 hour urines with 7,500 Fishman units of B-glucuronidase for 48 hours at 37°C, after adjusting to pH 4.5 and adding 2.5 ml of 1-M acetate buffer. The hydrolyzed urine was then acidified, extracted, and analyzed by the periodic acid method(9-11).

The cases studied represent 4 uncomplicated nephrosis patients and one recurrent nephritis patient. A. G. is a 42-year-old female, R B., a 49-year-old male, R. F., a 6-year-old male, T. K., a 5-year-old male, and B. R., the recurrent nephritic, is a 25-year-old male.

Results. Fig. 1 shows the glucuronidase activity of plasma (5 courses of treatment), leukocytes (3 courses), and urine (7 courses). In addition are given the data for the corticosteroids (total) in all the courses and the free corticosteroids in 2 of the courses of ACTH.

Plasma Glucuronidase: In all 5 courses studied for plasma glucuronidase activity, we are able to show a marked rise in the plasma glucuronidase level. In all cases, there is seen a distinct correlation between the plasma glucuronidase and the total urinary corticosteroid level. In the one case where the

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10. Cohen, S. L., *J. Biol. Chem.*, 1950, v184, 417.

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free corticosteroids are shown with the plasma glucuronidase, the plasma glucuronidase parallels this closely. The plasma activity, as well as the leukocyte activity, tends to sustain itself for several days after withdrawal of the ACTH.

Leukocyte Glucuronidase: In 2 of the 3 courses of ACTH studied, the white blood cell activity increases almost exactly proportionally to the rise in the plasma glucuronidase. In the third there is a temporary decrease followed by an increase in the enzyme activity.

Urinary Glucuronidase: Five of the 7 courses of ACTH on 5 patients show a definite rise in urinary B-glucuronidase. Two show no significant response. Both of these cases are children under 7 years of age. It cannot be said that there is any strict correlation between the urinary glucuronidase and the total corticosteroids. The corticosteroids increase rather uniformly during the treatment whereas the B-glucuronidase shows marked fluctuation. Nor can any correlation be discovered between the enzyme activity and the free corticoids as determined in 2 of the cases.

Total and Free Corticosteroids: All of the patients showed a rather uniform rise in total corticosteroids excreted during ACTH administration. The response on the peak day of corticosteroid output as compared with the control days varied individually from a 3-fold increase to a 15-fold increase.

In regard to the free corticosteroids as studied in 2 of the cases a little more can be said. In R. B. the clinical response was meager on the whole. However, the greatest decrease in cholesterol occurred during the time that the free corticosteroid level was at its peak. There was no appreciable diuresis in this case. In R. F. the marked increase in the free corticosteroid (5-fold increase) was accompanied by a considerable decrease in cholesterol and a major diuresis. In this respect the number of cases limits absolute correlation, but it can be said that the peak in free corticosteroid level occurred at the time of the most vigorous clinical response.

Discussion. Plasma and leukocyte B-glucuronidase values are subject to individual variation and since there are not, to our knowledge, any normal urinary values available, we are not able to say whether or not these fall within the normal range. However, on the days prior to ACTH administration the values obtained in the glucuronidase activity were fairly constant per individual.

The results seem to indicate that the action of the ACTH on the B-glucuronidase is a secondary one, contingent upon the increased output of the cortical hormones. If the action were a direct one it would be expected that the B-glucuronidase would increase before the corticosteroid rise whereas we found that the plasma and urinary glucuronidase increase paralleled or followed the corticosteroid increase.

From the data presented there is no evidence for believing that the B-glucuronidase mechanism accounts for the difference in clinical response and cortical response of a patient. Good response in the case of B. R. was accompanied by increased plasma and urinary enzyme activity while the relatively poor response in the case of R. B. resulted in a similar rise in the enzyme activity.

Summary and Conclusions. 1. Observations on the effects of activation of the adrenal cortex by ACTH on the B-glucuronidase activity of plasma, leukocytes, and urine are reported. 2. ACTH administration evokes a marked rise in plasma and white cell glucuronidase activity. Plasma glucuronidase activity correlates well with the total corticosteroid level elicited by the ACTH in that it increases during administration and decreases following administration although the day-to-day parallel is variable. 3. Urinary glucuronidase activity fails to indicate significant correlations either with plasma and white cell glucuronidase or with the corticosteroids.

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