Blood Adrenocorticotrophin in Children with Congenital Adrenal Hyperplasia.* (20222)

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The oxycellulose technic(1) for the analysis of ACTH in blood is now being applied to problems in pituitary-adrenocortical physiology. The preliminary data presented in this report suggest that the titer of ACTH is greater than normal in the blood of patients with adrenogenital syndrome.

Methods. 24 subjects were studied: 9 afebrile children without endocrine disease were included in the control group; 8 children (7 genetic females and 1 genetic male) with adrenogenital syndrome were included in a second group; and 7 children (3 with acute rheumatic fever, 2 with Sydenham's chorea, 1 with acute glomerulonephritis and 1 with idiopathic hypoglycemosis) were included in a miscellaneous group. A blood sample was obtained from either an internal jugular or an antecubital vein and added immediately to 4 volumes glacial acetic acid. The blood-glacial acetic acid mixture was processed by the oxycellulose‡ technic for the concentration of adrenocorticotrophin (ACTH)(1) and assayed in male hypophysectomized rats by the adrenal ascorbic acid-depletion method(2). In every assay, freshly prepared ACTH standard solutions were tested with blood eluates. The standard was assayed at 0.25 and 1.0 milliunit (mu§) or at 0.25, 0.5 and 1.0 mu per 100 g test rat. The eluate of a blood sample was divided so that a dose equivalent to 20, 40 or 50 ml of blood was injected into each of 2 to 5 assay rats.

Results. The individual and average responses of the test rats to given doses of blood together with the corresponding individual and average responses to the low dose of standard tested on the same day are presented in Table I.

The results are expressed in terms of adrenal ascorbic acid depletion, that is, the concentration of ascorbic acid in the left adrenal, removed prior to injection, minus the concentration of ascorbic acid in the right adrenal, removed one hour after injection of the test material. Negative values indicate that the concentration of ascorbic acid in the right adrenal of the test animal is higher than the concentration in the left adrenal. Fluctuations about zero for the difference between the concentration of ascorbic acid in the left and in the right adrenal following the injection of 0.9% sodium chloride solution represent, in part at least, the error of the analytical technics employed. Biological variation may also contribute to this fluctuation about zero. Results of an experiment in which 0.9% sodium chloride solution was injected follow: -40, 10, 7, -3, 8, 17, 12, 47, 12, 7, -13, -19, -12, 17, -18, 24, -11, -18, -15, -6, 24, 12, 2, 9, 30, -17, 9, 18, -10, average *3*.

In the control group, eluates equivalent to 20 to 50 ml of blood per test rat did not induce a significant depletion of adrenal ascorbic acid, whereas 0.25 mu ACTH per 100 g test rat induced a significant degree of depletion. Therefore, it can be stated that the concentration of ACTH in the blood of these control subjects appears to be less than 0.5 mu per 100 ml. Eluates equivalent to 40 ml blood obtained from untreated children with adrenogenital syndrome produced a significant depletion of adrenal ascorbic acid. Although the single-dose assays employed do not permit a

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§ One mu equals one-thousandth of a U.S.P. Unit of ACTH which is defined as the activity of one mg of the International Standard ACTH (La-1-A).

TABLE I. Blood ACTH in Children.

	Patient, sex and age (yr)	Dose (ml)	Blood sample Responses* Individual	Blood sample Responses* Avg	Standard ACTH 0.25 mu Responses* Individual	Standard ACTH 0.25 mu Responses* Avg
Control subjects	K.M. ♀ 4	20	−24, −25	−25	83, 66, 38, 54, 60, 111	69
	S.B. ♀ 3	40	−5, 18	7	83, 66, 38, 54, 60, 111	69
	L.S. ♀ 7	"	6, 18	12	27, 20, 35, 92, 50	45
	J.A. ♂ 3	20	5, 22	14	83, 66, 38, 54, 60, 111	69
	G.S. ♂ 9	40	−31, −16	−24	27, 20, 35, 92, 50	45
	L.S. ♂ 12	"	24, 14	19	66, 85, 66, 134, 91	88
	L.S. ♂ 8	"	−27, −26, 38	−5	22, 29, 25, 25, 105, 102	51
	L.M. ♂ 13	"	−30, −53, 36	−16	8, 12, 74, 11, 28, 63, 77, 22	37
	D.C. ♂ 9	"	−12, 26, 2	5	76, 85, 88, 111, 85	89
Adrenogenital syndrome prior to therapy	W.C. ♀ 4	20	29, 29, 29	29	83, 66, 38, 54, 60, 111	69
	S.Z. ♀ 9	40	76, 118, 69, 45	77	83, 30, 92, 54, 66, 68, 58	64
	F.P. ♀ 10	"	89, 20, 66	58	127, 102, 59, 114	101
	N.W. ♀ 4	"	62, 46, 84, 57	62	83, 30, 92, 54, 66, 68, 58	64
	J.C. ♀ 14	"	42, 56, 27	42	76, 85, 85, 111, 85	88
Adrenogenital syndrome during cortisone therapy	W.C. ♀ 4 (40)†	"	29, 6, −7, −10	5	27, 20, 35, 92, 50	45
	F.P. ♀ 10 (10)	"	−42, −28, −8, 23	−14	8, 12, 74, 11, 28, 63, 77, 22	37
	S.B. ♀ 3 (270)	"	−52, −15, −6, 3	−18	83, 30, 92, 54, 66, 68, 58	64
	E.Wi. ♀ 2½ (37)	"	32, −2, −42	−4	93, 83, 117, 84, 78, 76, 77	87
	E.Wi. ♀ 2½ (89)	20	46, −41, −48	−14	25, 19, 35, 25, 33	27
	R.Wi. ♂ 4 (33)	40	−40, 27, −44, −6	−16	93, 83, 117, 84, 78, 76, 77	87
	R.Wi. ♂ 4 (86)	20	11, 22, 23	19	25, 19, 35, 25, 33	27
Adrenogenital syndrome after cortisone therapy	R.W. ♀ 6 (60)‡	40	56, 18, 18, −20	18	66, 85, 66, 134, 91	88
	S.B. ♀ 3 (90)	"	−47, 19, 3, 51	7	8, 12, 74, 11, 28, 63, 77, 22	37
	E.Wi. ♀ 2½ (47)	20	75, 54, 41	57	37, 10, 21, 68, 90	45
	E.Wi. ♀ 2½ (30)	"	45, 52, 45	47	51, 93, 40, 16	50
	R.Wi. ♂ 4 (47)	"	5, 39, −17	9	37, 10, 21, 68, 90	45
	R.Wi. ♂ 4 (30)	"	−3, 12, 20	10	51, 93, 40, 16	50
Miscellaneous group	J.P. ♀ 8 (acute glomerulonephritis)	40	8, 8, 26	14	8, 12, 74, 11, 28, 68, 77, 22	37
	M.B. ♀ 4 (idiopathic hypoglycemosis)	"	47, 90	69	22, 29, 25, 25, 105, 102	51
	G.S. ♂ 9 (acute rh. fever without carditis)	"	16, −3, 38	17	8, 12, 74, 11, 28, 68, 77, 22	37
	D.S. ♂ 10 (acute rh. fever with carditis)	"	137, 132, 110	126	8, 12, 74, 11, 28, 68, 77, 22	37
	J.B. ♂ 14 (acute rh. fever with carditis—prior to cortisone Rx)	"	184, 155, 97	145	66, 85, 66, 134	88
	J.B. ♂ 14 (acute rh. fever with carditis—after cortisone Rx)	"	−37, −29, 8	−19	8, 12, 74, 11, 28, 68, 77, 22	37
	B.P. ♂ 14 (Sydenham's chorea)	"	−3, −4, −23, 12, −21	−8	50, 7, 33, 36, 41	33
	C.E. ♀ 8 (Sydenham's chorea)	"	−1, 8, 64	24	50, 7, 33, 36, 41	33

* Difference in ascorbic acid concentration (mg/100 g fresh adrenal) between left adrenal, removed prior to injection of test material and the right adrenal, removed one hr after injection of test material.
† Days on cortisone. ‡ Days after cortisone withdrawal.

definitive statement regarding the exact concentration of ACTH in the blood of these patients, they do indicate that a greater than normal concentration of hormone is present. Samples of blood obtained from children with adrenogenital syndrome during cortisone

therapy (12.5 to 50 mg/day, intramuscularly or orally) did not contain detectable quantities of ACTH when assayed at doses equivalent to 20 and 40 ml. ACTH was not detectable in the blood of patients following cortisone withdrawal except in the case of E. Wi. In the miscellaneous group, 2 of 3 children with acute rheumatic fever and a child with spontaneous hypoglycemosis had unusually high concentrations of blood ACTH. In the case of J. B., cortisone therapy was followed by a return of the blood ACTH titer to an undetectable level. These findings in patients with rheumatic fever are interesting and justify further investigation.

Discussion. It is to be emphasized that the data presented are insufficient to permit definitive conclusions. In particular, the study lacks individual cases in which blood samples were analyzed in all three categories, *i.e.*, prior to, during, and after cortisone therapy. This type of control study is important since the degree of involvement of the adrenal cortex undoubtedly varies among patients in regard to cortical hormone output.

Blood from children in the control group contained no detectable ACTH. It may be estimated that the concentration of ACTH is less than 0.5 mu per 100 ml blood. These findings are in agreement with observations in normal adults(3) and in non-stressed intact rats(3).

In the untreated children with adrenogenital syndrome ACTH was present in the blood in detectable quantities. The limited results are compatible with the hypothesis of Bartter *et al.*(4) and of Wilkins *et al.*(5) that in the adrenogenital syndrome there is an increased rate of discharge of ACTH from the pituitary [See schema presented in Fig. 13 of the paper of Bartter *et al.*(4)]. The increased activity of the adenohypophysis is presumed to be secondary to a primary defect in the adrenal cortex. The hyperplastic adrenal gland produces an excess quantity of androgen but a relatively deficient quantity of cortical hormone. That the adrenal cortex is incapable of producing an optimal quantity of cortical hormone is suggested by the fact that the administration of ACTH fails to induce a significant increase in the titer of blood 17-hydroxycorticosteriods(6). Furthermore, the reduction in blood ACTH titer by cortisone is compatible with the concept that the primary defect is in the target gland and not in the adenohypophysis.

Blood ACTH was detectable in only one patient with adrenogenital syndrome in the post-cortisone group. This is difficult to explain in terms of the above concept since one would expect the titer of ACTH to rise following cortisone withdrawal. However, the dose of cortisone, the duration of therapy, and the time elapsed after withdrawal of therapy are variables which must be investigated in greater detail before definitive conclusions are made.

Summary. The oxycellulose technic has been applied to the analysis of blood ACTH in children. ACTH was not present in detectable quantities in the blood of afebrile children without endocrine disease. ACTH was detected in the blood of untreated, but not of cortisone-treated, children with adrenogenital syndrome.

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