BLOOD GLUTATHIONE LEVELS AND CARBOHYDRATE TOLERANCE DURING A.C.T.H. THERAPY

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Many biochemical changes in the body following the administration of adrenocorticotrophic hormone (A.C.T.H.) have been described, and these have been studied extensively by numerous workers (see Sprague, 1951). Among them, Conn, Louis, and Wheeler (1948) have reported decreased carbohydrate tolerance, increased uric acid excretion, and a fall in the blood glutathione (GSH) level. They concluded that a metabolic state similar to that in diabetes mellitus had been produced, and that a permanently diabetic condition might result if the administration of A.C.T.H. was continued. Both Conn *et al.* (1948) and Lazarow (1949) speculate on how these changes are produced, and it has been suggested that the fall in the blood GSH level may be due, as in experimental alloxan diabetes, to the presence of some circulating substance which reacts not only with the blood GSH, but also with thiol groups in the β -cells of the pancreas, thereby producing its diabetogenic effects. Attention has also been drawn to the production of experimental diabetes in rabbits by the administration of uric acid (Griffiths, 1948). Conn, Louis, and Johnston (1949) have further claimed that A.C.T.H.-induced hyperglycaemia can be abolished by the administration of GSH. In a study of three patients with rheumatoid arthritis undergoing A.C.T.H. therapy Sprague *et al.* (1950) were unable to confirm the results of Conn *et al.* (1948). While the latter authors' experimental conditions were not exactly fulfilled, no significant changes in GSH levels were noted during treatment.

The GSH levels quoted by Conn *et al.* (1948) in support of their conclusions do not appear to be consistently or markedly abnormal, and it was thought that the relationship between carbohydrate intolerance and GSH levels in the blood during A.C.T.H. administration needed further clarification; this point has also been stressed by Sprague (1951). The levels of blood GSH have therefore been studied in five patients receiving "routine" (I.V.) A.C.T.H. treatment, and in a further group of six patients who were given A.C.T.H. by the same route and in the same dosage as that used by Conn *et al.* (1948).

Methods and Material

Blood glutathione levels have been estimated by two methods: in Cases 1, 3, 4, and 5 duplicate determinations were made both by the iodometric method of Woodward and Fry (1932) and by a modification (Thompson and Watson, 1952) of the nitroprusside method of Brückmann and Wertheimer (1947). The nitroprusside method gives results that consistently are slightly lower than those obtained iodometrically, but as this difference is small, and in view of the close parallelism (see Graph) between the results obtained by the two methods, in the last seven cases blood GSH levels have been measured by the nitroprusside method only. In all cases estimations were made on protein-free filtrates obtained from freshly drawn blood (oxalated or heparinized). Estimations of the packed cell volume were made on all samples.

Relationship between GSH levels obtained by the nitroprusside and iodometric methods.

Observations were made upon 11 patients who had been recommended for A.C.T.H. on therapeutic grounds, and who were seriously ill. Two of them (Cases 6 and 7) had generalized pemphigus of such severity that without A.C.T.H. they would probably have died. Case 9 had moderately severe pemphigus confined to the buccal mucous membrane. Six other patients (Cases 1, 2, 3, 4, 10, and 11) had rheumatoid arthritis, Case 10 being complicated by sarcoidosis and Case 11 by psoriasis. The disease was in all cases severe enough to interfere with their lives, but only Case 11 was bedridden. One severe case of rheumatic fever (Case 8) and one of advanced scleroderma (Case 5) were also included.

Administration

Studies were first made upon five patients who received the following standard course of A.C.T.H. in use in this hospital: on the first and second days, 40 mg. daily, given over eight hours in an intravenous infusion of 540 ml. of 5% glucose-saline; on the third and fourth days, 30 mg. daily, similarly administered; subsequently, 20-25 mg. daily, similarly administered, for about 18 days, with further reductions in dosage in the last days of the course.

By using the intravenous instead of the intramuscular route a great economy in the amount of A.C.T.H. used is made (Duthie, 1952), but difficulties inevitably arise during the course of daily intravenous transfusions, necessitating occasional changes to the intramuscular route, with consequent variations in effective dosage. For this reason, and because of the desirability of reproducing the conditions of Conn *et al.* (1948) as exactly as possible, further studies were made on six patients who each received 50 mg. A.C.T.H. intramuscularly three times daily for eight days, and then small and rapidly decreasing dosages, at first intravenously and then intramuscularly, to avoid the dangerous "rebound" phenomena which have occasionally been observed to follow abrupt cessation of high dosage (Duthie, 1952). In the latter group early morning urine specimens were tested daily for sugar during the course and for a short time before and after it. A glucose-tolerance test was made before starting A.C.T.H. and again on the sixth day of treatment, this day being chosen as Conn *et al.* (1948) found the lowest GSH levels on the fourth, fifth, and sixth days of treatment. The results of these observations are presented in Table I.

Blood GSH levels were estimated on most days, except over the week-ends and for short periods before and after the course of A.C.T.H. These results are presented in Table II. It should be noted that GSH values recorded on day 0 in this Table were obtained before the administration of A.C.T.H.

Results

Response to treatment was judged so far as was possible by objective criteria. In the cases of pemphigus, disappearance of the skin lesions and their failure to recur during treatment was convincing proof of effect. The rheumatoid syndromes and the case of scleroderma were assessed by increase in the range of movement of affected joints.

TABLE I.—Levels of Blood Sugar Obtained During Glucose-tolerance Tests (a) Before and (b) on the Sixth Day of A.C.T.H. Therapy, and the Appearance of Glycosuria in Fasting Specimens and During these Tests

Case	Blood Sugar (mg./100 ml.)						Glycosuria	
	0	30 min.	60 min.	90 min.	120 min.	150 min.	Fasting	During G.T.T.
6 { a	57	101	135	120	114	148	Nil	—
6 { b	111	123	193	232	212	—		
7 { a	74	105	—	163	128	185	"	—
7 { b	50	69	150	200	180	180		
8 { a	79	93	155	130	118	96	"	Nil
8 { b	92	174	208	142	100	108		
9 { a	60	85	109	115	97	55	Present	"
9 { b	82	135	124	105	87	75		
10 { a	78	128	—	111	105	75	Nil	Nil
10 { b	98	123	137	114	95	—		
11 { a	82	122	122	102	97	94	"	"
11 { b	100	187	234	—	196	76		

Improvement in the case of rheumatic fever was shown by the disappearance of symptoms, settling of the fever and pulse rate, and fall in the E.S.R. from about 60 mm. to about 10 mm. in one hour (Westergren). In passing, it was noted that the degree of so-called "A.C.T.H. euphoria" roughly paralleled these objective criteria. In view of the criticisms levelled against eosinophil counts (Rud, 1947) they are not here recorded.

As six good and three fair clinical responses were seen, and the two poor results were both in unpromising subjects, there seems to be no reason to doubt the potency of the A.C.T.H. that was used.

A.C.T.H., as was observed by Conn *et al.* (1948). In the present series, however, it was never of such severity as to cause the fasting glycosuria that was noted by them, and three of our four cases who showed such intolerance had a "lag" type of blood-sugar curve. It is evident that the degree of carbohydrate intolerance induced in these cases was not so great as that seen by Conn *et al.* (1948). From the therapeutic results there is no reason to doubt the potency of the A.C.T.H., although the possibility must remain that the material used in this work was less active than that employed by Conn. Further, all the present patients were seriously ill, as also were those studied by Sprague *et al.* (1950), whereas Conn's were healthy subjects.

In none of these cases, nor in five others, was the level of blood GSH significantly reduced during A.C.T.H. administration, and no relationship between blood GSH levels and carbohydrate intolerance could be established. This latter finding does not support Conn's hypothesis.

It is quite clear, however, that some degree of carbohydrate intolerance is induced by the administration of A.C.T.H. in some cases by some means. At least one patient has developed permanent diabetes following a course of A.C.T.H. (Glyn, 1951), and persistent hyperglycaemia and glycosuria have been induced in rats by its administration (Ingle, Li, and Evans, 1946).

It does not seem, therefore, that the decreased carbohydrate tolerance induced by A.C.T.H. is regularly accompanied by a detectable fall in the blood GSH level; these results do not support the view that an alloxan-like action initiates the development of transient diabetic states.

TABLE II.—Glutathione Levels Obtained Before, During, and After A.C.T.H. Therapy. For Details of Administration and Dosage see Text. Each Course began on Day 0, and the Day of Ending is Indicated by a Bracket

No.	Diagnosis	Sev- erity	Levels of Glutathione (mg./100 ml. Blood)															Res- ponse				
1	Rh. arthritis	Int.	29.5	—	—	26.2	29.0	28.8	28.5	27.6	—	—	29.5	24.0	28.5	27.4	28.5	26.5	—	—	—	Fair
2	"	"	34.0	35.8	35.0	37.7	36.5	37.0	—	42.2	—	42.0	38.0	37.5	—	—	35.5	38.8	36.0	—	—	Good
3	"	Adv.	35.2	—	—	32.0	33.4	36.6	34.8	31.8	—	—	33.0	29.5	28.4	34.6	—	—	—	—	—	Fair
4	"	Int.	—	—	29.0	31.4	32.1	36.6	—	—	26.4	27.2	29.1	31.0	32.8	—	—	32.0	34.2	33.2	—	Good
5	Scleroderma	Adv.	—	—	—	31.2	—	—	38.2	—	—	31.0	—	—	29.8	—	—	—	29.5	29.5	—	Poor
6	Pemphigus	"	—	29.8	26.5	27.6	—	—	25.4	27.5	29.8	27.0	31.0	—	—	—	29.5	—	—	—	—	Good
7	"	"	21.8	—	21.4	—	20.0	—	19.2	—	20.7	20.0	23.5	—	—	—	24.0	—	—	—	—	Good
8	Rh. fever	Sev.	29.5	31.0	27.8	—	—	30.6	27.7	—	26.1	28.3	—	—	—	25.5	24.5	25.4	26.6	—	—	"
9	Pemphigus	Mod.	—	33.0	30.0	35.0	34.8	—	—	31.5	30.2	31.6	34.5	33.9	—	—	—	31.5	30.0	—	—	"
10	Sarcoidosis	Adv.	—	44.0	41.6	46.0	41.5	—	—	44.0	41.2	47.0	43.0	41.2	—	—	42.2	44.0	—	—	—	Fair
11	Psoriasis arth- ropathica	"	—	31.8	32.6	—	—	32.0	28.8	30.4	36.5	28.0	—	—	28.5	27.8	32.6	—	—	—	—	Poor
	Day No.	..	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	

Studies of carbohydrate tolerance were made in the last six cases, each of which received 150 mg. A.C.T.H. intramuscularly daily. From Table I it will be seen that Cases 6, 7, 8, and 11 showed some degree of carbohydrate intolerance during treatment. In Cases 6, 8, and 11 the values suggested a "lag" type of curve, but in Case 7 results more typical of true diabetes were seen. In Cases 6 and 7 the very extensive skin lesions made the collection of urine samples during glucose-tolerance tests impossible, but glycosuria was seen in Cases 8 and 11 during the second glucose-tolerance test. Case 9 was found to have a low renal threshold before A.C.T.H. was given. In no other case was glycosuria found in the fasting specimens, and these results therefore give no support to Kass, Ingbar, and Finland (1950), who have suggested that the renal threshold for sugar is lowered by A.C.T.H.

The results in Table II were obtained from frequent estimations of blood GSH levels by the nitroprusside method. It will be seen that there is no significant variation in these levels before, during, or after A.C.T.H. administration, although good therapeutic effects were observed.

Discussion

From the above results it is clear that carbohydrate intolerance can be induced by the administration of

I wish to express my thanks for advice and encouragement to Professor R. H. S. Thompson, at whose suggestion this work was undertaken, and in whose department the estimations of blood glutathione levels were made. I am also grateful to Mr. G. Clough and to Miss M. de Jong for their technical assistance. Sufficient A.C.T.H. to treat the last six cases was made available through the courtesy of the Medical Research Council, to whom I am most grateful.

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