TABLE I. Smallest Inoculum Required to Initiate Growth of Anaerobes.

Organism	Anaerobic device: Inoc. in large arm	Anaerobic device: Inoc. in small arm	Brewer's thioglycollate medium
Cl. perfringens	10^{-6}	10^{-9}	10^{-8}
Cl. lentoputrescens	10^{-6}	10^{-10}	10^{-10}
Cl. butyricum	10^{-4}	10^{-6}	10^{-7}
Cl. sporogenes	10^{-6}	10^{-8}	10^{-9}
Cl. tetani	10^{-1}	10^{-6}	10^{-6}
Cl. novyi	10^{-1}	10^{-3}	10^{-3}
Cl. bifermentans	10^{-1}	10^{-9}	10^{-9}
Cl. histolyticum	10^{-5}	10^{-11}	10^{-7}
Cl. septicum	10^{-4}	10^{-5}	10^{-6}
Cl. botulinum	10^{-4}	10^{-11}	10^{-8}

Serial dilutions of a heavy broth culture of the strains were made in 0.85% NaCl solution, so that the indicated inocula, in terms of original broth culture, were contained in 0.1 ml. The anaerobic device contained approximately 20 ml of broth of which 15 ml were in the small arm. Ten ml of Brewer's medium were used for testing. The endpoint was the smallest inoculum which produced visible growth on incubation.

rubber gasket, the gas may readily be removed by syringe and needle or other apparatus for analysis.

Motile variants of bacterial organisms may be isolated from mixed cultures by means of the device. The tubes are filled with semisolid medium and an inoculum is placed near the surface of one arm. During incubation the motile variants grow down the inoculated arm, through the capillary and up the opposite arm to the surface, where they may be recovered readily.

The anaerobic culture device can be made by a skillful glassblower by using Kimble Neutraglass culture tubes with plastic caps (No. 45066) and Kimble Neutraglass capillary tubing (N-51A, No. 46486), outside diameter 7 to 8 mm. The gaskets can be punched from rubber sheeting (heavy inner tubing will do) by means of a cork borer of appropriate size.

Summary. A simple device is described by means of which anaerobic bacteria can be grown in liquid medium without the addition of chemical reducing agents or pieces of tissue, and without the use of an anaerobic jar or similar apparatus.

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Response to Adrenocorticotrophic Hormone in Clinical Scurvy.* (18250)

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A close relationship between ascorbic acid metabolism and adrenal cortical function is suggested by evidence derived chiefly from animal experiments(1-3). Diminished adrenal cortical activity might be expected, therefore, in human ascorbic acid deficiency. Accordingly, the change of the blood eosinophils and urinary uric acid following test doses of adrenocorticotrophic hormone (ACTH) was measured in 5 patients with clinical and laboratory evidence of scurvy before and after the administration of therapeutic amounts of ascorbic acid. Serum sodium and potassium concentrations likewise were measured before and after the specific antiscorbutic therapy.

Each of the 5 patients had typical scurvy as evidenced by perifollicular hemorrhages, ecchymoses, especially of the posterior thighs and buttocks, a positive tourniquet test, and

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1. Selye, H., Textbook of Endocrinology, Acta Endocrinoligica, 1947, Montreal University, Montreal, Canada.

2. Long, C. N. H. Recent Progress in Hormone Research, I, 1946, Academic Press, New York.

3. Sayers, G., and Sayers, M. A., *Ann. N. Y., Ac. Sc.*, 1949, v50, 522.

TABLE I. Effect of Test Doses of ACTH in Clinical Scurvy Before and After Ascorbic Acid Therapy.

Patient Age	W.W. 66	F.O'D. 74	J.C. 67	W.C. 36	P.C. 75
Days between tests	22	20	12	*	7
Duration ascorbic acid therapy before 2nd test (days)	11	11	7	0	2
Total dose ascorbic acid (g)	13	11	7	0	6
Ascorbic acid content of buffy coat (mg/100 cc)					
1st test	0	4	3.4	0	0
2nd " ‡	37	23.7	10	—	0
4-hr eosinophil response† (cells/cu mm)					
1st test	115/35	0/0	210/67	57/4	150/31
2nd " ‡	64/10	17/10	289/84	—	144/47
Uric acid excretion† (mg/4 hr)					
1st test	84/49	—	86/44	14/17	143/66
2nd " ‡	65/10	60/43	40/78	—	131/65
Blood glutathione (mg/100 cc)					
1st test	25	20	35	26	12
2nd " ‡	22	21	—	—	15
Blood pressure (mm Hg)					
1st test	95/50	120/70	150/95	138/80	130/70
2nd " ‡	100/55	100/55	150/95	—	120/60
Serum sodium (mEq/L)					
1st test	140.3	129.4	138.5	147.5	—
2nd " ‡	138.5	134.7	143.5	—	—
Serum potassium (mEq/L)					
1st test	4.8	4.8	4.5	—	—
2nd " ‡	4.1	4.3	4.2	—	—

* Expired after 6 days of study.
† Tests before/4 hr after ACTH administration.
‡ After ascorbic acid.

a low buffy coat ascorbic acid content. Blood coagulation studies, including platelet counts, were normal.‡ Four of the patients gave dietary histories of prolonged ascorbic acid deficiency. The other patient (J.C.) had eaten tomatoes at irregular intervals. He also had a urinary tract infection. The interrelationship of the dietary and disease factors in this case is not clear, nevertheless clinical scurvy was present. All patients were examined within a few hours after hospital admission and were immediately transferred to the Thorndike metabolic ward. None of the patients was given ascorbic acid as such or in food prior to the study. During the period of study they were offered a diet consisting of boiled rice and milk, crackers, sugar, and coffee. This diet contained minimal quantities of ascorbic acid. One vial of ACTH§ was used for each test. The hormone was dissolved in saline and administered intramuscularly. Since other metabolic studies were concurrently conducted, the patients were not given ascorbic acid for from 5 to 11 days after admission. An ACTH test was done in all patients in the scorbutic state and repeated in 4 of these after the intravenous or oral administration of from 6 to 13 g of ascorbic acid. One patient expired on the sixth day of the study. Permission for autopsy was not obtainable.

Buffy coat ascorbic acid concentration was measured by the method of Butler and Cushman, which utilizes the reduction of 2-6 dichlorophenolindophenol(4). Urine uric acid was determined by the method described by Benedict and Franke(5). The blood eosinophil and urine uric acid investigations were conducted as outlined by Thorn, Forsham,

‡ Carried out through the kindness of Dr. Frederick S. Bigelow.

§ Provided by Dr. John R. Mote, The Armour Laboratories, Chicago 9, Ill. (Assayed to contain 35 mg of activity per vial compared to the standard).

4. Butler, A., and Cushman, M., *J. Clin. Invest.*, 1940, v19, 459.

5. Benedict, S. R., and Franke, E., *J. Biol. Chem.*, 1922, v52, 387.

Prunty, and Hills(6). Blood glutathione was determined according to the method of Woodward and Fry(7). Serum sodium and potassium concentrations were analyzed by flame photometry.

Results. The essential data are presented in Table I. In all patients prior to therapy, buffy coat ascorbic acid was absent (minimal dye reduction, probably of no significance, was observed in 2 instances). Following the administration of ascorbic acid 2 patients showed a return of the buffy coat ascorbic acid to normal and a definite but less marked increase occurred in one patient. Another patient (P.C.) who had been given less ascorbic acid over a shorter period of time than the others, showed no change. The reason for this is not evident since 4 g of ascorbic acid are usually enough to restore the ascorbic acid content of the buffy coat of a scorbutic patient to or near to normal(8). Following the administration of ascorbic acid, the tourniquet test was negative and ecchymoses were diminishing in all patients, including P. C.

The eosinophil response to ACTH in each instance was as great or greater than that considered indicative of normal adrenal activity, except for patient F. O'D. This patient had no circulating eosinophils prior to ascorbic acid administration. The administration of ascorbic acid did not alter significantly the control eosinophil counts nor the responses to ACTH in any of the patients studied.

An increase in the urinary excretion of uric acid after ACTH occurred in only one instance, in fact a decrease was most often observed. This paradoxical response is probably related to the low urine outputs which occurred in the 4 hours after the hormone, despite the ingestion of water. The ACTH possibly contained antidiuretic activity sufficient to account for this.¶

The blood glutathione concentration was either normal (25 to 35 mg per 100 cc) or slightly below normal in all instances in which the determination was done, except one patient (P.C.) whose concentration was low. There was no significant change in glutathione concentration following ascorbic acid administration. Since blood glutathione is largely confined to the red cells, alterations in red cell mass might be expected to influence whole blood glutathione concentration. Although hematocrits were below normal in 4 of the 5 patients, there was no correlation with the glutathione concentration, either before or after ascorbic acid administration.

Serum sodium concentration was normal in 3 patients in whom the determination was done, and low in one patient (F. O'D.). The administration of ascorbic acid did not appreciably alter these values. Normal serum potassium concentrations were found in the 3 patients in whom it was measured before and after ascorbic acid therapy.

Discussion. Four patients with clinical and laboratory evidence typical of scurvy had normal eosinophil responses to ACTH. This evidence of normal adrenal cortical function is further supported by the finding of initially normal eosinophil counts in these 4 patients and by the absence of eosinophils in the fifth patient with florid scurvy. Diminished adrenal cortical activity, as in Addison's disease, is accompanied by a high eosinophil count, a reduced serum sodium, an elevated serum potassium, and hypotension. In addition to normal eosinophil responses to ACTH, 3 of these patients with scurvy had normal serum sodium and 3 normal potassium concentrations prior to ascorbic acid therapy. Hypotension was not a prominent or consistent finding in these patients. The urine uric acid excretion after ACTH was not interpretable, probably because of the low urinary outputs after hormone administration. According to present concepts, the normal eosinophil response to ACTH and the failure to find consistent hypotension, hyponatremia, and hyperkaliemia, indicate

6. Thorn, G. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. B., *J.A.M.A.*, 1948, v137, 1005.

7. Woodward, G. E., and Fry, E. G., *J. Biol. Chem.*, 1932, v97, 465.

8. Lewis, J. H., and Davidson, C. S., unpublished observations.

¶ The lot of ACTH used was reported to contain, on different assays, between 0.14 and 1.3 units of pressor activity.

normal adrenal cortical function in these patients with scurvy. The observation, however, is amenable to other interpretations. First, ascorbic acid may not be necessary for those functions of the adrenal cortex studied. This interpretation is supported by the report of Long(9) that the injection of ACTH to scorbutic guinea pigs with but 4% of normal adrenal ascorbic acid remaining resulted in the usual fall in adrenal cholesterol and the development of lymphopenia. Secondly, although scorbutic, the patients studied may have had residual ascorbic acid in their adrenals.

It is impossible to decide between these alternatives at present. It is known, however, that scorbutic guinea pigs retain small but significant quantities of ascorbic acid in their adrenals(10). The same may be true in human scurvy. Moreover, patients dying of scurvy sometimes present a syndrome of hypotension and shock resembling an Addisonian crisis(11). Perhaps only terminally do the adrenals become completely deficient in ascorbic acid.

Summary. 1. Test doses of ACTH were administered to 5 patients with classical scurvy to assess their adrenal cortical activity. The test was repeated in 4 of these patients after therapy with ascorbic acid. 2. The blood eosinophil response to ACTH was that expected of individuals with normal adrenal function and did not change following treatment with ascorbic acid. 3. The urinary excretion of uric acid after ACTH was uninterpretable because of low urine volumes. 4. The blood glutathione, and the serum sodium concentrations were normal or low in these patients, but were unaltered by ascorbic acid therapy. Serum potassium concentrations were normal before and after ascorbic acid therapy. 5. To explain these evidences of normal adrenal cortical activity in scurvy, it may be postulated that either ascorbic acid is not necessary for these adrenal functions, or there was residual ascorbic acid in the adrenals of these patients.

Addenda. Dr. Frank H. Gardner, Peter Bent Brigham Hospital, made a similar study of one patient, a 76-year-old bachelor, with scurvy. A 48-hour ACTH test‖ was done, giving 40 mg of ACTH daily, divided into 4 equal doses. A normal eosinophil response and increase in 17-ketosteroid excretion was observed.

	Control	48 hr
Eosinophil count per cu mm	27	0
17-ketosteroid excretion mg/24 hr	2.3	13.6

The patient presented in addition to clinical evidence of scurvy, the defect in tyrosine metabolism reported in the disease.**

9. Long, C. N. H., *Fed. Proc.*, 1947, v6, 461.

10. Robertson, W. V., Dalton, A. J., and Heston, W. E., *J. Nat. Cancer Inst.*, 1949, v10, 53.

11. Gardner, F. H., personal communication.

‖ Thorn, G. W., and Forsham, P. H. Clinical Tests for Adrenal Cortical Reserve. Progress in Clinical Endocrinology, 1950, Grune & Stratton, New York.

** Rogers, W. F., and Gardner, F. H., *J. Lab. and Clin. Med.*, 1949, v34, 1491.

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Failure of Aureomycin and Terramycin to Increase Resistance to Toxic Action of Influenza Virus.* (18251)

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Acute toxic damage is induced by the influenza virus in tissues which will not support its multiplication(1). When injected intravenously, intra-abdominally or intra-

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