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Received October 14, 1952. P.S.E.B.M., 1952, v81.

Fecal Electrolytes and Nitrogen During Cortisone or ACTH Therapy.*† (19905)

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In view of the established effects of cortisone, ACTH, and DOCA on the excretion of certain body constituents through the kidney and the skin(1-6), the possible influence of two of these agents on the composition of stools has been studied in a series of patients. Compound E, 50 to 1000 mg per day, or ACTH, 10 to 100 mg per day administered for 3 to 50 or more days, did not produce any characteristic alteration in the output of electrolytes and nitrogen. Collection of control data to establish this fact has incidentally provided information about the amounts of sodium, potassium, chloride and nitrogen excreted in feces during maintenance of a miscellaneous group of subjects on certain types of milk diets.

Materials and methods. Stools have been collected from hospitalized children and adults while on one of the following dietary regimens supplemented by vitamins and iron: a) essentially complete sodium restriction, *i.e.*, low-sodium milk fortified with carbohydrate and milk protein, providing an adequate nitrogen and caloric intake, or b) a whole milk diet similarly modified. Patients with gastrointestinal disorders were excluded, but the available clinical material made it impossible to limit the study to any one category of patients with respect to age or disease. This study is therefore based upon analyses of stools from a miscelleneous group of children and adults ill with rheumatic fever, diabetes mellitus, rheumatoid arthritis, leukemia, muscular disorders, *et cetera*. The daily intake of formula usually exceeded two liters but the seriously ill patients at times took less than the prescribed amount.

The *output* of sodium, potassium, chloride and of nitrogen in formed stools has been measured in control studies as well as during periods of therapy with cortisone or ACTH. Water was taken as desired. The beginning and end of a stool collection period, usually

* Aided by grants from the W. Pa. Chapter of American Rheumatism Assn., the Damon Runyon Memorial Fund, and the U. S. Public Health Service.

3 to 7 days in length, were marked by administering carmine red, 0.3-0.6 g, in a gelatin capsule. Stools colored red by the first capsule were either discarded, or if a part of a series, assigned to the preceding interval, whereas those marked by the second dose of the dye were added to the current collection. Particular care was taken to exclude any unformed or diarrheal stools as well as those contaminated with urine. During the collection period and thereafter the specimens were refrigerated in tared and stoppered bottles. Following determination of the stool weight approximately 200 ml of concentrated sulfuric acid was added to each specimen. They were stirred and permitted to digest overnight. Distilled water was then added in amounts sufficient to produce a homogenous thin suspension with the aid of either a Waring blendor or an Osterizer and the final volume measured to establish the dilution factor. Immediately thereafter duplicate or multiple aliquots of the suspended stool up to 6 in number were pipetted into appropriate vessels for processing and analyses. The procedure developed by Wallace was used in preparing the specimens for sodium and potassium determinations(7,8). Approximately 3 ml of concentrated nitric acid were added to the beaker containing the aliquot of suspended stool together with sufficient distilled water to make a final volume of less than 100 ml. The mixture was then boiled for ten minutes, cooled, diluted to 100 ml and filtered. Suitable dilutions to which lithium sulfate had been added were then analyzed on a Barkley internal standard flame photometer with an experimental error of 2% or less(8). The Hald modification of the silver nitrate method was employed for the stool chloride analyses (9). The procedure was simplified in the later studies by allowing the digestion to occur in tubes placed in boiling water for 30 minutes. In those instances in which an excess of potassium permanganate had been introduced superoxol was added and the boiling repeated. Comparison of this modification with the usual digestion of individual tubes over an open flame indicated that the accuracy of the method was unaffected. The stool nitrogen was measured by means of the macro-Kjeldahl technic in which duplicate analyses usually agree within 2%(10). *Stool electrolyte excretion* has been expressed in milliequivalents per day, in milliequivalents per 100 g of wet stool, and in milliequivalents per g of stool nitrogen. Nitrogen excretion has in turn been presented in grams per day and per 100 g of stool mass. Inspection of the results of analyses of stools collected prior to or following hormone therapy with those obtained during therapy in the same individuals revealed no trends in any of the constituents under study. The results have therefore been pooled in accordance with the dietary and treatment regimens which have been outlined and submitted to conventional statistical treatment. After exclusion of the one or two values more than 3 S.D. away from the mean present in most of the categories, the mean and S.D. were obtained in the usual manner. A "p" value of less than 0.05 was taken to represent a statistically significant difference.

Results. From Tables I and II it is readily evident on comparison with control values that cortisone and ACTH, alone or in combination, exerted little or no influence on the composition of the feces. The statistically significant changes recorded in stool nitrogen and chloride in the low-sodium milk group receiving cortisone and in stool chloride in those treated with ACTH (Table I) are of negligible magnitude.

Inspection of the control data in Tables I and II reveals that during either regimen the fecal potassium is approximately 5-fold greater than the sodium, and that the excretions of these 2 ions are of the same order of magnitude irrespective of the milk formulae used. The stool chloride is somewhat greater on whole milk in which considerably more chloride is present. The rate of loss of nitrogen in feces is the same in each group, averaging 0.8 g per day and slightly more per 100 g of stool.

Discussion. Cortisone and ACTH did not alter the output of electrolytes or of nitrogen in this group of miscellaneous patients. This means of course that the gastrointestinal tract, at least in terms of its ultimate output, does not respond, as do the kidneys or the sweat glands, by a diminished sodium output and a

TABLE I. Stool Electrolytes and Nitrogen on a "Sodium-Free Milk Formula"† Without and With ACTH or Cortisone Therapy.

Therapy		Na			K			Cl			N	
		meq./d.	meq./100 g	meq./l.	meq./d.	meq./100 g	meq./l.	meq./d.	meq./100 g	meq./l.	g/d.	g/100 g
0	Mean	1.3	2.3	2.3	8.1	12.3	12.2	.7	.8	.9	.8	.9
	No.	44	48	46	47	48	46	46	45	44	48	46
	S.D.	1.1	2.1	2.1	5.3	8.2	7.7	.7	.5	.6	.4	.3
Cortisone	Mean	1.3	1.6	2	10.7	12.8	11.5	.5	.6	.5*	1	1.2*
	No.	27	26	25	25	26	22	24	24	21	25	24
	S.D.	.6	.7	1.5	7	5.1	4.5	.5	.5	.5	.8	.4
ACTH	Mean	1.5	1.6	1.6	8.7	8.5	10.9	1.4*	1.4*	1.8*	.8	1
	No.	16	15	11	16	14	11	13	11	9	11	11
	S.D.	1.1	1.1	1.1	5.7	5	7.1	1.4	1.1	1.4	.5	.3
Cortisone + ACTH	Mean	.8	1.8	1.2	7.8	18.2	16.6	.6	.6	.4	.7	1.2*
	No.	6	6	6	6	6	6	3	3	3	6	6
	S.D.	.8	1.3	1.2	4.6	12.4	13				.6	.4

* "p" values less than .05.

† Composition of low-sodium milk formula: Na = 1.1 meq./l; K = 44.9 meq./l; Cl = 18.5 meq./l; N = 5.3 g/l.

TABLE II. Stool Electrolytes and Nitrogen on a Whole Milk Formula* Without and With Cortisone Therapy.

Therapy		Na			K			Cl			N	
		meq./d.	meq./100 g	meq./l.	meq./d.	meq./100 g	meq./l.	meq./d.	meq./100 g	meq./l.	g/d.	g/100 g
0	Mean	1.9	2.3	2.4	12.2	14.6	14.6	1.3	1.2	1.4	.8	1
	No.	38	38	35	40	38	34	38	37	36	38	38
	S.D.	1.4	1.6	1.4	6.8	7	5	1.3	.7	1	.5	.4
Cortisone	Mean	1.7	2.3	2.9	10	13.9	18	.7	.9	1.1	.7	.9
	No.	15	14	15	15	14	15	14	13	12	15	13
	S.D.	.8	1.1	1.7	5.7	6	8.8	.5	.6	.8	.6	.2

* Composition of whole milk formula (based on 30 analyses): Na = 26 meq./l; K = 37 meq./l; Cl = 31 meq./l; N = 5.4 g/l.

greater loss of potassium. It should be pointed out however that our data do not obviate the possibility of very marked effects of adrenocorticotropic hormone or cortisone on electrolytes and nitrogen in the upper portions of this tract with subsequent cancellation of these changes in the lower bowel. As a matter of fact indirect evidence has been presented indicating that in clinical situations characterized by undue sodium retention, exchange resins may not exert their maximal effect(11). It has been postulated that this reflects an increase in adrenocortical activity or effects.

Summary and conclusions. 1. Cortisone and adrenocorticotropic hormone did not produce any characteristic change in fecal electrolytes and nitrogen in a group of miscellaneous patients maintained on milk formulae of restricted or unrestricted sodium content. 2. Data have been presented on the fecal excretion of sodium, potassium, chloride, and nitrogen during maintenance of such patients on a whole milk or sodium-free milk formula.

The authors wish to express their indebtedness to J. Finlay, E. Seitz, R. Schneider, and C. Berg for their assistance in preparing the stools for analyses.

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Received October 15, 1952. P.S.E.B.M., 1952, v81.

Factors Related to Growth of Psittacosis Virus (Strain 6BC) III. Uracil and Related Compounds.* (19906)

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Previous observations(1-3) indicate that pteroylglutamic acid and or citrovorum factor are required for the intracellular proliferation of psittacosis virus and that certain purines (adenine, guanine) and other compounds are important for the intracellular multiplication of psittacosis virus (6BC). Since these various substances are concerned with the intracellular synthesis of nucleic acids, of which desoxyribonucleic acid (DNA) has been shown to be a prominent constituent of the psittacosis group of viruses(4), it was of interest to see if evidence could be obtained that the pyrimidine uracil, a material substituent of ribonucleic acid (RNA), is related to the intracellular proliferation of psittacosis virus.

Methods. Tissue cultures were prepared by planting whole minced tissues of 9- to 11-day-old chick embryos on perforated cellophane discs(5) in the bottoms of 10 ml Erlenmeyer flasks(2). Two ml of nutrient solution composed of 2 volumes of Hanks's(6) balanced solution and one volume of ox serum ultrafiltrate, to which virus was added, were then placed into each flask. The psittacosis virus (6BC) had been cultivated in the yolk sacs of embryonated eggs for a long period of time. Tissue cultures were incubated at 36°C. After 24 hours the infected nutrient fluid was withdrawn and replaced with 2 ml of fresh nutrient solution to which one of the various compounds had been added. The compound was added to the fluids for 2 successive changes after which normal nutrient solution was employed. Fluid changes were carried out every 4 days and the fluid removed was promptly tested for its virus content by injection into embryonated eggs. A single dilution of the virus-containing fluid from each tissue culture was prepared in broth and 0.25 ml of the material was injected into the yolk sacs of 12 or more 7-day-old embryonated eggs. Eggs were candled daily, deaths recorded, and the average day of death calculated. The virus titer was determined from this value by the method described(7).

To evaluate the possible toxic action of the test compounds on chick embryo tissues, 8

* This investigation was supported by a research grant from the National Institutes of Health, Public Health Service, and by a contract between the Office of Naval Research, Department of the Navy, and the University of Rochester.