tion of streptolysin O, which is a protein. Surprisingly, hemagglutination titers were identical with a variety of human sera when these two preparations were used as sensitizing antigens. (2) Hemagglutination was shown by many sera in titers ranging from 1:4 to 1:128, but there was no correlation between the magnitude of antistreptolysin and hemagglutination titers. Patients with non-streptococcal respiratory infections, streptococcal pharyngitis, and acute rheumatic fever could not be differentiated on the basis of the hemagglutination test. (3) The one consistent observation was that, for any one patient, hemagglutination titers tended to remain constant with both acute and convalescent sera. This suggests that a cross reaction of some type was responsible for the agglutination of red cells sensitized with streptococcal products. Further study is necessary to clarify the nature of this phenomenon.

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B Vitamin Content of Rat Adrenals with Respect to Exposure to Cold. (19126)

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Sayers(5) has ably reviewed the biochemical changes observed in the adrenal gland under stress. While it has been noted that stress stimuli effect the depletion of ascorbic acid(6) and cholesterol(7) and the turnover of phosphorus(2) in the adrenal cortex, no data are available to indicate whether other substances, particularly other vitamins, are similarly affected. There is presented here the results of a study of the comparative concentration of four B-vitamins (biotin, folic acid, niacin and riboflavin) in the adrenals of rats before and after subjection to cold stress for one hour at 5°C.

Materials and methods. Female rats of the Holtzman strain, weighing approximately 150 g, were used. All had access to a stock diet of N.C.I. food pellets(3) and tap water *ad libitum*, supplemented weekly with 0.2 ml administered cod liver oil. Twenty rats constituted each experimental group. Ascorbic acid was determined by the method of Roe and Kuether(4) as described by Sayers(8) with modifications in temperature and time of incubation as outlined in the procedure below. The B-vitamins were liberated enzymatically, as recommended by Cheldelin(1), with papain and takadiastase. They were assayed microbiologically with *L. casei* in 2 ml volume, employing a medium essentially similar to that described by Tepley and Elvehjem(9) with appropriate supplements and omissions for adaptation to the several vitamin assays. The rats were anesthetized with ether, the left adrenals were removed and weighed to the nearest milligram and dropped immediately into McShan homogenizers containing one ml

* U. S. Public Health Service, Federal Security Agency.

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TABLE I. Vitamin Content of Rat Adrenals Before and After Exposure to Cold.

Series	State	Ascorbic	Biotin	Folic	Niacin	Riboflavin
A	Before cold	3.54 ± .376	.469 ± .028	1.52 ± .129	80.4 ± 13.5	27.5 ± 3.20
	After cold*	2.20 ± .360	.465 ± .025	1.31 ± .132	68.5 ± 5.9	33.5 ± 3.33
B	Before cold	3.01 ± .470	.518 ± .039	1.29 ± .170	63.4 ± 7.6	29.7 ± 2.57
	After cold*	1.90 ± .164	.524 ± .031	1.57 ± .353	70.1 ± 7.5	31.1 ± 3.39

* Exposure for 1 hr at 5°C.

Ascorbic acid values expressed as mg per g of fresh adrenal tissue; all other values as μg per g. Each series includes 10 pre-exposure and 10 post-exposure determinations on the paired adrenal glands of 20 rats.

acetate buffer, pH 4.7. Two left adrenals, and later, 2 right adrenals, were pooled to provide a sample weight of 35-40 mg. After operation and recovery from anesthesia, each pair of rats was confined in a plastic cage placed in a cold room at 5°C for one hour. They were then killed with ether and the right adrenals taken and similarly treated.

As soon as each group of 20 was completed (*ca* 2 hours) the adrenals were homogenized and sufficient buffer added to make a 1:100 suspension. One ml of this suspension was pipetted into a test tube (19 x 150 mm) containing 9 ml trichloracetic acid for the ascorbic acid assay. The remaining suspension was transferred to 19 x 155 mm test tubes and one drop each of 2% papain and 2% takadiastase preparation added. The tubes were then stoppered and placed in an incubator at 37.5°C for 24 hours.

Ascorbic acid assay. Reagents. Trichloracetic acid, 4%; Norit A, Pfanstiehl; dinitrophenylhydrazine reagent, 2% in 9 N H_2SO_4; with 0.25% thiourea added, then filtered; sulfuric acid, 85% (9:1).

Procedure. One ml of homogenate in 9 ml of trichloracetic acid reagent was treated with 0.3 g Norit with shaking for a few moments, then filtered through a dry filter. Four ml of the filtrates were pipetted into colorimeter tubes, one ml of dinitrophenylhydrazine reagent added with mixing and the tubes were placed in a water bath at 60°C for 75 minutes. The tubes were then transferred to an ice bath and 5 ml of 85% sulfuric acid added to each, dropwise, followed by mixing. The tubes were removed from the ice bath and permitted to stand for about 20 minutes in order to come up to room temperature. They were read in a Coleman Universal Spectrophotometer at 540 millimicrons. Ascorbic acid was computed by reference to a semilog graph for standard solutions covering the working range (0-20 μg) and similarly treated.

B vitamin assay. The reagents and media have been identified above; dl-alanine was omitted in all cases and peptone was included only in the riboflavin assay. Each liter of stock media was supplemented with 20 μg of folic acid except for that used in the folic acid assay.

Procedure. Dilutions of the enzymatic digest were made to provide a 1:2000 concentration for biotin and folic acid, 1:1000 for niacin and 1:500 for riboflavin. Assays were conducted in duplicate at three levels of substrate (0.2, 0.6 and 1.0 ml) plus one ml of media. Distilled water was added to make a total of 2 ml in 13 x 100 mm tubes. The tubes were capped and sterilized at 15 lb pressure for 15 minutes. When cool, they were inoculated with one drop each of a saline suspension of thrice-washed *L. casei* cells from a 24-hour broth culture. Low blanks were assured by preparing the suspension in a dilution at least 50 times that of the original broth inoculum. After approximately 66 hours incubation at 37.5°C the cultures were titrated by 0.1 N NaOH with bromthymol blue as indicator. Vitamins were computed by reference to standard curves prepared in the usual manner.

Results and discussion. It will be seen from the data presented in Table I that the conditions of these experiments were such as to afford an extensive ascorbic acid depletion in the stressed animals. Thus the ascorbic depletion in Series A is 38% and in Series B it is 37%. In marked contrast, the changes noted in the content of biotin, folic acid,

niacin, and riboflavin are very limited and within the expected variability of the microbiological methods employed. These observations lend increased specificity to the ascorbic acid depletion effect since they indicate that other water soluble factors, which are also intimately involved in cellular metabolism, are not as readily altered by stress.

The concentration of ascorbic acid in the adrenal is very high in comparison with other tissues of the body(4). The observed adrenal concentrations of biotin, folic acid, niacin and riboflavin are of about the same order as previously found for rat liver and kidney(1). This contrast also serves to emphasize the special significance of the high adrenal content of ascorbic acid.

Summary. In contrast with the expected extensive depletion of ascorbic acid, the biotin, folic acid, niacin and riboflavin content of the rat adrenal gland was observed not to be materially altered by exposure to cold.

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Effect of Starvation on Exchangeable Potassium and Tissue K^{42} Content in Rabbits.*† (19127)

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In the course of a clinical survey of individuals with various diseased states, low values for exchangeable potassium, as measured by the radioactive isotopic technic, were encountered(1). In general, these low values appeared to be due to: (1) a decrease in body mass secondary to increased catabolism produced by the disease process; (2) a diminished intake of food from anorexia induced by the illness; (3) an intracellular deficiency of this cation from some inherent metabolic abnormality; or to a combination of these factors. The role of human disease and of metabolic abnormalities would be best studied in patients who present such problems. The effect on potassium metabolism of a decrease in food intake in the absence of disease can be approached experimentally. Since such a study was not feasible on human beings in our laboratory, rabbits were starved and the changes produced were followed serially.

The purpose of the present study is to determine the effect of starvation for a short period of time on the total potassium content of the body, as well as its concentration in various tissues. Because of the greater correlation of weight and urinary creatinine with exchangeable potassium content in human males as compared with females, the following experiments were performed in male animals (2,3).

Material. Nineteen normal male adult rabbits of mixed breeds were used. They were placed in individual metabolism cages and were fed a stock diet of compressed pellets. Tap water was given without restriction.

Methods. Experimental procedure. The experiment was planned to study the alterations in potassium metabolism which would be produced by a reduction in body weight of ap-

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