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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proximately 15%. A loss in weight of this extent is not uncommonly encountered clinically and would be comparable to a decrease of 12.5 kg (22.5 lb) in a 70 kg (150 lb) man. Because of the technical difficulties involved in the use of an isotope with such a short half-life (12.4 hours), an interval of one week was found to be convenient between isotopic determinations. Group A consisted of 7 animals which were observed for a control period of one week before starvation and subsequently during the deprivation of food. External potassium balance studies were conducted for the baseline period and the first week of starvation. At the conclusion of this period the animals were still in good condition, so that the observations with radiopotassium were continued for an additional week, but balance studies were not done. Each morning before feeding, the body weight, total food consumption and the daily urine volume were recorded. Creatinine determinations were performed in the morning on an aliquot of 24 hour daily sample. Baseline exchangeable potassium determinations were completed in the morning just before food was withdrawn. This determination was repeated after 1 and 2 weeks of starvation. Six rabbits (Group B) were fed for a period of one week to establish equilibrium on the diet. Food was then withdrawn completely, though water was allowed *ad libitum*. After one week the animals were sacrificed by exsanguination and analyses for radiopotassium and total water content were performed on 17 different tissues. Another group of 6 animals (Group C) served as a control for Group B. These rabbits were continued on food and water *ad libitum* but otherwise were treated identically with Group B.

Radioisotope methods. The preparation of the potassium solution for injection has been previously described(2). A calibrated volume of 5 ml of the K^{39} solution containing 30 μc of K^{42} in 0.5 milliequivalent was injected intravenously. The urine was collected for 24 hours; a spot specimen was then obtained by catheterization and its specific activity was determined. The following formula was used to calculate the value for the *exchangeable potassium content* of the body:

$$Ke = \frac{K_i^{42} - K_o^{42}}{\frac{Ku^{42}}{Ku^{39}}}.$$

K_e = quantity of exchangeable K in milliequivalents.

K_i^{42} = quantity of radiopotassium administered (arbitrary units).

K_o^{42} = quantity of radiopotassium excreted in urine until spot specimen was obtained.

Ku^{42}/Ku^{39} = specific activity of spot specimen.

Tissue analyses. Three aliquots (1 to 3 g) of each tissue were obtained. They were kept in covered Petri dishes and were weighed as rapidly as possible on a Gramatic balance to 0.1 mg. Two portions were digested in concentrated sulfuric acid for determination of *radiopotassium concentration.* The digest was made up to a volume of 20 ml and counted with a dipping tube and a scaling circuit. The tissue concentration of radiopotassium was expressed as per cent of the injected dose per g of wet tissue. *Tissue water content* was determined on the third aliquot, which was dried to constant weight at 110°C for 18-24 hours. The *potassium* concentration in the urine was determined by flame spectrophotometry by a method which has been previously described (4). Urinary *creatinine* concentration was determined by a modification of the method of Bonsnes and Taussky(5). *External balance of potassium.* Each g of feed was found to contain 0.159 meq. of potassium. The tap water contained a negligible amount. The potassium content of the ingested feed was found to be completely absorbed through the gastrointestinal tract. The content of potassium in the stool was too low to be detected by flame photometry. The loss of potassium from the body was, therefore, calculated on the basis of its urinary excretion. *Statistical method.* The significance of the difference between the means was derived by the use of the "t" test(6).

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Results. The 7 animals followed by external balance and K_e determinations (Group A) were in potassium balance and gained weight during the baseline period (Table I, Fig. 1). During the period of starvation a progressive loss of body weight occurred. The mean weight loss for the group after the first week was 0.35 kg (15.6% of the mean initial weight) (Table I, Fig. 1); this amount, however, was not statistically significant. After 2 weeks, the mean decrease in weight amounted to 0.71 kg, which is significant. At the end of the first week the mean negative potassium balance amounted to 23 meq. The mean K_e decreased 41 meq. during the first week and an additional 34 meq. during the second week of starvation. By the end of the second week of starvation, a significant reduction in K_e/wt had occurred.

Although the mean creatinine excretion decreased 146 mg during the first week and 124 mg during the second week, as compared with the baseline values, these changes, as well as the decreases in the creatinine coefficient, were not significant. The urine volume increased the first week of starvation.

Discussion. The present results emphasize the importance of the concept that the body potassium is in dynamic equilibrium, and that the changes in the rate of exchange may be the first indication of a disturbance in its metabolism. Such an abnormality may occur before any alteration in concentration of potassium in blood or tissue is detectable.

During starvation, as tissue is catabolized, the weight decreases progressively and the total body content of exchangeable potassium decreases. The continued loss of potassium in the urine during starvation confirms the endogenous source of this cation. With tissue catabolism, the K_e to weight ratio decreases at a relatively constant rate, at least for the first two weeks of starvation.

The exchangeable potassium loss after a week of starvation exceeds by 18 meq. the potassium loss as measured by the external urinary balance method. According to the method of calculation of K_e, the more in-

TABLE I. Effects of Starvation on Potassium and Creatinine Metabolism in Normal Male Rabbits (Group A).

Starvation	Wt, kg	Exchangeable K (meq)	Ke/wt (mg/kg)	Urinary creatinine (mg/wk)	Creatinine coef. (mg/kg)	Urine vol (ml/wk)	Oral intake K (meq/wk)	Urinary K^{39} excretion (meq/wk)
Before	2.24 ± .33*	137.4 ± 15.4	62 ± 5.43	694 ± 207	45.8 ± 4.85	901 ± 356	77.8	76.6
1st wk	1.89 ± .33	96.4 ± 23	51.8 ± 14.80	548 ± 155	38.9 ± 7.95	1456 ± 165	0	23
2nd wk	1.53 ± .35	62.4 ± 11.7	41.4 ± 4.45	424 ± 160	34.8 ± 7.92	1149 ± 355	0	—

* Standard deviation.

Results of Balance Studies

Starvation	wt	Exchangeable K (meq)	Ke/wt (meq/kg)	Urinary creatinine (mg/wk)	Creatinine coef. (mg/kg)	Urine vol (ml/wk)	Urinary K^{39} excretion (meq/wk)
1st wk	—.35	—41*	—10.2	—146	— 6.9	+555*	+23
2nd wk	—.71*	—75*	—20.6*	—270	—11	+248	—

* Statistically significant difference when compared with baseline values. P = <.01.

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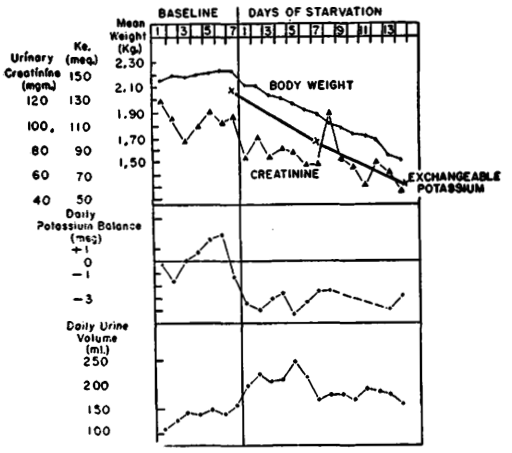

FIG. 1. The effect of starvation on potassium and creatinine metabolism in normal male rabbits.

complete the exchange of radioactive atoms with the normal intracellular untagged potassium ions, the smaller would be the calculated exchangeable potassium content. In normal rabbits approximately 90% of the body potassium would have exchanged in 24 hours(2). It appears that with starvation and the consequent reduction in metabolic rate, which is known to set in quickly during fasting, the rate of turnover of intracellular potassium is decreased, or the relative amount of "bound" potassium is increased.

Comparison of the values in the normal animals (Group C) and the rabbits starved for one week (Group B) revealed no significant difference in the concentrations of tissue water or tissue radiopotassium for the following tissues: skin, subcutaneous connective tissue, abdominal muscle, gastrocnemius muscle, quadriceps muscle, diaphragm, tendon, cartilage, heart ventricle, heart auricle, lung, liver, spleen, stomach, appendix, adrenal and kidney. The mean weight loss of the starved animals after one week of starvation was 0.43 kg (13.8% of the initial mean body weight). Since the tissue analyses show no significant difference, it is possible, therefore, to account for the total decrease in body potassium on the basis of tissue breakdown without postulating an intracellular deficiency in the remaining tissues.

The creatinine coefficient serves as an index of relative muscle mass. Although the decrease in this ratio observed with starvation may imply 1) a decrease in the rate of metabolism of muscle tissue; 2) a decrease in the total muscle mass, or both, the differences at one and 2 weeks were not significant when compared with the baseline value. With diminished intake of food, a relatively greater utilization of tissue fat and carbohydrate in preference to protein would be expected initially. The utilization of fat was evident grossly at the time the samples for analyses were obtained; the amount of perirenal fat in the starved animals, for instance, was considerably less than in the control rabbits, though the muscles appeared similar.

Since no significant change in radiopotassium concentration was found in any specific tissue or organ in starved animals, the decrease in rate probably was generalized over the body. The possibility that such an alteration might be limited to the skeletal or nervous system must be considered, since these two tissues were not included in the present analyses. The radioactive potassium method, therefore, appears to be a more sensitive index of the functional defect produced by starvation than the external balance method.

The possibility that the loss of potassium in the feces during starvation was increased over the normal amount must also be considered. In such an event, the urinary balance study alone would underestimate the total loss. However, since, during the prestarvation period, there was no loss in the stool, it would be highly unlikely that the discrepancy of 18 meq. between the external urinary balance and the K_e could be explained on this basis alone.

These studies complement our clinical observations and lend support to the hypothesis that whenever catabolic processes exceed the anabolic, the loss of body potassium, weight, and muscle tissue parallel each other(1).

Summary. 1. Male rabbits were starved in order to study the effect of a decrease in food intake on potassium metabolism. 2. Serial determinations of the exchangeable potassium content and urinary potassium and creatinine excretion were made in one group of animals. The mean decrease in exchangeable potassium

content was 29.8 and 54.6% of the baseline value after one and two weeks respectively. The corresponding mean decrease was 15.6 and 31.7% in body weight. No significant changes in urinary creatinine excretion occurred. 3. In a separate group of rabbits, tissue analyses for radiopotassium and water concentration after one week of starvation revealed no significant changes when compared with control animals, continued on a constant diet. 4. The decrease in total body potassium content, as measured by the exchange of isotopic potassium, as well as the loss in the urine during starvation could be accounted for on the basis of tissue catabolism without postulating an intracellular deficiency of this cation in the remaining tissues. A functional abnormality in potassium metabolism may be detectable by the radioisotope method prior to its manifestation by the external balance method.

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Cholic Acid: An Adequate Stimulus for Hypercholesteremia in the Normal Fasting Rat.* (19128)

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Recent studies(1,2) in this laboratory have indicated that the administration of sodium cholate to a rat with biliary obstruction markedly increases the degree of hypercholesteremia ordinarily occurring after obstruction. This effect of cholate could be obtained as well in the starved as in the fed animal. Moreover, parenteral injection of cholate was as effective as oral administration of the drug.

These facts suggested to us the possibility that perhaps intravenous administration of cholate to the normal, intact, starved rat might in itself produce hypercholesteremia. The present report indicates the results of such administration of cholate.

Methods. Male rats (Long Evans) approximately 10 weeks of age, were operated upon under ether anesthesia. One end of a flexible polyethylene cannula (diameter: 0.011 inch) was inserted into the inferior vena cava of each animal via a lumbar tributary. The other end of the cannula was attached to a syringe whose piston was gear driven in a special apparatus† at such a rate as to deliver 0.3 ml of fluid per hour. The abdominal incision was then closed and the rats were placed in individual Bollman cages(3). In this manner, a preparation was obtained which allowed the *continuous* intravenous injection of fluid into unanesthetized rats. Six of the animals were given sodium cholate in Tyrode's solution at a rate equivalent to 25 mg of cholic acid per hour. The remaining eight rats received Tyrode's solution, alone, thus serving as controls. Each of the experimental rats received approximately 500 mg of cholate per day. The rats were starved for 24 hours before operation and during the experiment. Blood samples taken before and 24 hours after the beginning of the injection were analyzed for bile acids and cholesterol in plasma according to previously described methods(4,5).

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† The entire apparatus was constructed for us by Charles Calvert, Pacific Industrial Electronics Co., San Francisco, Calif.

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