Effect of Dietary Protein Restriction on Plasma Volume, "Available (Thiocyanate) Volume" and Muscle Water. (18260)

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In the past, the majority of workers have supported the theory that the production of "malnutritional edema" is closely associated with hypoproteinemia. On the other hand, various more recent authors have doubted the importance of hypoproteinemia in this respect. Thus, the results of the experiments by Weech, Snelling and Goettsch(1) and by McClure and Hinman(2) were taken to give strong support to the view that a correlation exists between the fall of the plasma protein concentration and the accumulation of edema fluid. In contrast, Henschel, Mickelsen, Taylor and Keys(3) concluded that though hypoproteinemia may play a role, "the edema in simple caloric starvation is largely a reflection of a reduction in cellular mass without large change in the absolute amount of extracellular fluid."

The present experiments were carried out on rats and dogs maintained on protein-deficient diets. The study was undertaken to determine whether changes of the "available (thiocyanate) volume" ("SCN") and of the skeletal muscle chloride space held any relation to each other and to the extent of the plasma protein deficit.

Methods. In the experiments on rats, young adult females, weighing between 200 and 258 g, were placed on a control diet(4) or on a diet containing fresh raw carrots as the only source of protein. Vitamin supplementation was made orally as in previous experiments(4). Rats on the carrot diet were sacrificed at the end of 2 weeks, and at the end of succeeding 2-week periods through 14 weeks. In the experiments on dogs, young adult animals were placed on a constant diet(5) for a period of at least 7 days before the experiments were undertaken. The majority of the dogs received for 3 weeks a protein-free diet, differing from the control diet only in glucose substitution for the casein. In the case of 4 dogs the dietary period was extended to 74 days. Plasma volume (P.V.) and "SCN" were determined on dogs by the direct method of Gregersen and Stewart(6) as adapted to the photoelectric colorimeter by Gibson and Evelyn(7). The method was modified slightly when applied to rats. The collection, sampling and chemical analyses of the blood and skeletal muscle were carried out as previously described(4,5). The extracellular and intracellular water partition of skeletal muscle was calculated in the manner outlined by Hastings and Eichelberger(8).

Results and discussion. Plasma volume and "available (thiocyanate) volume."* From the data presented in Table I it is seen that in rats with plasma albumin deficit the P.V. tended to be lowered when expressed per unit of control weight, and tended to be increased when calculated per unit of experimental

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* None of the dogs receiving the protein-free diet for the 3-week period exhibited clinical edema, while one of the 4 dogs given the diet for 74 days showed a small amount of peritoneal fluid when the experiment was terminated. Of 12 rats placed on the carrot diet for 10 weeks or longer, 6 exhibited some fluid in the peritoneal cavity when they were sacrificed.

TABLE I. Average Plasma Albumin and "Fluid" Volume Changes in Rats and Dogs Receiving Protein-Deficient Diets.*

Body wt†	Plasma albumin, g/100 ml	Plasma vol. as ml		"SCN" vol. as ml		Ratio: "SCN" / P.V.	Skeletal muscle per kg fat-free tissue			
		Per control wt§	Per exper. wt§	Per control wt§	Per exper. wt§		H_2O g	Cl mEq	Extracell. H_2O as g	Intracell. H_2O as g‖
Rats receiving carrot diet.										
222(15)	2.81	4.08		26.3		6.5	762.4(6)	10.5	95	738
178(2)	2.56	3.55	4.33	24.3	30.0	6.9	765.5	12.3	113	736
162(7)	2.38	3.18	4.58	20.3	29.1	6.4	771.0	14.2	130	738
142(5)	2.07	2.83	4.80	17.4	29.5	6.2	770.1	19.2	172	724
143(9)	1.89	3.30	5.34	19.4	31.4	5.9	773.5	18.8	175	727
131(3)	1.65	3.57	6.06	20.7	35.0	5.8	784.3	28.0	244	718
Dogs receiving protein-free diet.										
11.8(82)	3.40	47.1		293		6.3	758.7(5)	11.7	96	733
9.4(5)	2.85	40.0	42.4	266	282	6.7				
9.1(4)	2.62	39.2	41.5	255	272	6.5				
8.9(6)	2.33	41.8	45.7	278	303	6.7				
9.6(7)	2.08	42.0	46.6	305	338	7.3				
10.1(3)	1.96	44.6	47.8	347	371	7.8				
9.0(4)‡	1.86	32.5	51.8	215	342	6.7	776.8	18.2	144	741

* Data represent averages grouped according to extent of plasma albumin deficit.
† Exp. weight, given as g for rats and as kg for dogs.
‡ Maintained on diet for 74 days and data tabulated separately.
§ Per 100 g in the case of rats and per kg in the case of dogs.
‖ Per 1000 g of muscle cells.

weight. The "SCN" in these animals was found to be changed in a similar manner and consequently the ratio of "SCN": P.V. was not increased. In general, similar findings were obtained in the dogs placed on the protein-free diet. However, the ratio of "SCN": P.V. tended to be increased in those dogs given the diet for 3 weeks and exhibiting a marked reduction of plasma albumin. On the other hand, the average ratio of "SCN" : P.V. was not appreciably different from the control value in those dogs placed on the protein-free diet for 74 days despite an even greater reduction of the plasma albumin concentration.

The results of the present experiments are quite comparable to those obtained in a study(3) of normal men subsisting on an European type of famine diet for an extended period of time. The "SCN" calculated per unit of experimental weight indicated the presence of a relative excess of extracellular fluid even though there was a decrease in the total "SCN."

Skeletal muscle water and chloride space. From the average data presented in Table I, it will be seen that in rats the muscle water and chloride contents progressively increased as the plasma albumin concentration was reduced. The extracellular water content calculated on the basis of muscle chloride was increased while the intracellular water per 1000 g muscle cells remained relatively unchanged. Similar changes were observed in the skeletal muscle of the 4 dogs placed on the protein-free diet for 74 days.

The relation between the extracellular water content of muscle and the plasma albumin deficit noted here is similar to that observed by McClure and Hinman(2) who used muscle sodium as the basis for calculation of the extracellular phase. Recently, in rats receiving a protein-deficient diet or undergoing starvation, Dicker(9,10) observed an increase of the skeletal muscle chloride space which could be detected in spite of a normal plasma protein concentration. When considered by itself the increase in the extracellular water of muscle calculated from muscle chloride (or sodium) can be interpreted to indicate an accumulation of total extracellular water. However, the simultaneous determination of the "SCN" revealed a loss or a tendency toward a stabilization, rather than an increase, of total extracellular fluid in animals placed

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on a protein-deficient diet and exhibiting hypoproteinemia. Such a situation can be interpreted along the lines expressed by Henschel, Mickelsen, Taylor and Keys(3) as representing a reduction of cellular mass without a large change in the absolute amount of extracellular fluid.

Summary. Hypoproteinemia induced in rats and dogs by protein-deficient diets was accompanied by a fall of the P.V. and "SCN" when expressed per unit of control weight and increased when expressed per unit of experimental weight. Thus, there was a decrease in the absolute extracellular volume, as measured by thiocyanate, in the presence of a "relative" extracellular hydration. The extracellular phase of muscle calculated on the basis of the muscle chloride content progressively increased as the plasma albumin decreased. This can be interpreted as indicating an accumulation of extracellular fluid accompanying a progressive hypoproteinemia. However, because of the observed decrease of the "SCN," these observations appear better explained on the basis of a reduction in cellular mass without a large change in the absolute amount of extracellular fluid.

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Protective Action of Antibiotics Against the Toxin of *Pasteurella pestis* in Mice. (18261)

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Many investigators(1-4) have pointed out the antitoxic activity of penicillin and the sulfonamides against various bacterial toxins. Both penicillin and aureomycin exhibit antitoxic activity against certain strains of the psittacosis-lymphogranuloma venereum microparasites(5) and aureomycin, against some viral and rickettsial toxins(6). Penicillin and the sulfonamides, however, offer little or no protection against toxins of *Pasteurella pestis*(7). Because more success in experimental therapy has been achieved with other antibiotics(8,9), the following experimental work was instigated.

Exp. 1. *Protection with Aureomycin, Terramycin and Chloramphenicol.* The toxins were obtained by extracting a 2% suspension of acetone-killed and dried, agar-grown *Pasteurella pestis* (Yreka) in physiologic saline. The bacteria were removed by centrifugation. To test the protective value of aureomycin, terramycin and chloramphenicol, 1.0 ml of a solution containing 2 mg/ml of the antibiotic was injected intraperitoneally into albino (Swiss) mice. Four hours later, 0.5 ml of a serial dilution of the toxic solution was injected intravenously into groups of 10 mice each. A control group of mice was given 1.0 ml of physiologic saline. Results are presented in Table I.

Exp. 2. *Duration of protection.* In this series, the period between giving the antibiotic and giving the toxin was varied to determine how long the protection lasts. The same amount of the drug was given in each dose by the same route; 1, 12 and/or 24 hours later, plague toxin (2 LD_{50}) was given intra-

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