phore hormone content in pigmented rabbits varies in the anterior + intermediate lobes between 33-150 frog units and in the posterior lobes between 15-150 frog units. In albino rabbits the values found amounted to 42-150 frog units in the anterior + intermediate lobes and to 15-500 frog units in the posterior lobes. Thus it is evident that the albino animal produces chromatophore hormone at least to the same extent as the normally pigmented rabbit and there is no significant difference in the pituitary content of this hormone between either type of animal.

Summary. The chromatophore hormone content of the pituitaries of pigmented and of albinotic rabbits has been examined in tree frogs (*Hyla arborea*). There is no significant difference between the two types of animals, the hormone content in the anterior + intermediate lobes in pigmented animals amounting to 30-150 frog units and in albinotic animals to 42-150 frog units. In the posterior lobes the values for pigmented animals were between 15 and 150 frog units and for albinotic animals between 15 and 500 frog units. We conclude that the only difference between the albinotic and the normally pigmented individual is the lack of enzymes of the type of DOPA oxidase or tyrosinase. This fact should be borne in mind in the approach to the treatment of vitiligo.

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Effect of Large Loads of Sodium on Bone and Soft Tissue Composition.* (23624)

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Recent data from this laboratory have demonstrated that in acute sodium depletion, approximately 70% of the sodium removed from the body is derived from the extracellular phase, 25% from bone mineral, and only 5% from the intracellular compartment(1). In view of these findings it was decided to determine whether bone could also act as an acceptor of sodium ions, under conditions of prolonged sodium-loading, or whether excess sodium would be retained in the extracellular fluid or the body cells. Therefore, a small group of rats was given a daily sodium load approximating 50% of their total body sodium. After 24 days they were sacrificed and the sodium content of bones and soft tissues determined. It was found that this procedure caused an increase of 8.6% in bone mineral sodium, associated with a 24% loss of bone water. These changes in bone occurred in the absence of changes in body weight, extracellular fluid composition, or the composition

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of soft tissues as exemplified by muscle and liver.

Methods and calculations. Two groups of 400 g male albino rats (3 control, 5 experimental) were placed on a diet of Purina rat checkers, with fluid *ad lib.*, for a period of 24 days. The rats were of identical age, one year and one week, and had been raised under identical conditions for 10 months preceding the experiment. Their weight had been stable for 2 months. These precautions were observed so as to eliminate from the experiment those changes in bone water and sodium which are known to occur during growth(2). The experimental rats received 0.85% sodium chloride as drinking water, and in addition were given 1 ml of 3% sodium chloride/100 g of body weight, intraperitoneally, 5 days each week for a total of 19 doses. At sacrifice, the animals were anaesthetized with intraperitoneal pentobarbital (5 mg/100 g body weight), exsanguinated through the abdominal aorta, and samples of muscle, liver, and bone obtained. Analyses of these tissues for water, neutral fat, sodium, potassium and chloride were made by methods previously described by the authors(1,3). Plasma was analyzed for water, sodium, potassium, and chloride. In addition, total phosphate was measured in muscle by methods previously reported(1,4) and calcium in bone by a modification of Bergstrom's method(5).

The size of the extracellular space in muscle and bone was estimated using the assumption that all the chloride in the tissue sample was extracellular(6). Plasma ultrafiltrate concentrations of electrolytes were calculated from their concentrations in plasma water, using a Donnan distribution factor of 0.96(7). Bone mineral, used as the reference base for bone water and electrolytes, was assumed to constitute two-thirds of the fat-free dry solids of both control and experimental animals(8).

Analyses of the liver in the rat yield sodium:chloride ratios which are smaller than those of plasma, indicating that some or all of the cells in rat liver contain chloride (7). Because of this, the distribution of chloride cannot be used to measure the size of the extra-cellular space in this tissue. Accordingly, the following calculation was devised in order to estimate this space in rat liver. It was assumed that 1) intracellular sodium exists at the same concentration in the cells of liver as in muscle, and 2) that the intracellular water of rat liver approximates 500 cc/kg of fat-free liver(4). The amount of sodium present in this intracellular water was then subtracted from the total amount of sodium found in the tissue, and a "sodium space" calculated from the remainder of the sodium. Such a calculation, while obviously not exact, gives values for intracellular liver potassium concentration which closely approximate those found in the muscles and red cells of the rat(15) and values obtained in rabbit liver using a chloride space(4).

TABLE I. Plasma Composition in Control and Sodium-loaded Rats.

	pH	H_2O, g/l	Na	K	Cl
			⌐ meq/l plasma ¬		
Control	7.53	944	140	3.7	105
	7.48	943	144	4.3	105
	7.50	948	141	3.9	104
Avg	7.50	945	142	4.0	105
Na-loaded	7.57	949	140	3.8	104
	7.51	956	140	3.6	105
	7.41	950	140	4.0	106
	7.48	951	139	3.7	100
	7.49	954	140	3.8	102
Avg	7.49	952	140	3.8	104
p	>.5	>.10	>.10	>.5	>.5

Statistical comparisons of the data from the 2 groups of animals were made using Student's t-test, incorporating Bessel's correction for small samples.

Results. The control rats had an average weight of 385 g at the beginning of the experiment and 394 at the end. The average weight of the sodium-loaded rats rose from 405 to 409 g. There was no significant difference between the 2 groups, and no excessive weight gain in the experimental animals.

Tables I and II present the values obtained on analysis of plasma, muscle, liver, and bone in the 2 groups of animals. Despite the heavy sodium load, plasma composition remained unchanged in the experimental animals, with the exception of a slight statistically insignificant increase in the plasma water.

TABLE II. Tissue Composition in Control and Sodium-loaded Rats.

	H_2O, g	Na, meq	K, meq	Cl, meq	PO_4, mM	Ca, mM
	(units/100 g fat-free dry solids)					
Control muscle	310	7.9	46.9	4.8	31.6	
	313	7.5	46.0	4.8	30.6	
	315	8.5	46.4	4.8	28.5	
Avg	313	8.0	46.4	4.8	30.2	
Muscle Na-loaded	331	8.4	45.6	5.2	29.4	
	314	7.9	46.2	5.2	29.3	
	322	6.8	48.7	4.9	30.4	
	317	7.6	44.0	5.1	29.8	
	326	9.5	45.8	4.9	29.7	
Avg	322	8.0	46.1	5.1	29.7	
Control liver	242	8.8	31.1	9.0	36.4	
	233	8.3	33.7	9.4	34.4	
	245	8.4	33.0	8.6	37.8	
Avg	240	8.5	32.6	9.0	36.2	
Liver Na-loaded	257	8.5	33.4	10.3	38.4	
	237	7.6	34.1	9.2	36.1	
	245	8.5	36.9	9.8	38.1	
	234	7.7	32.3	9.1	34.9	
	257	7.7	35.4	10.4	40.0	
Avg	246	8.1	34.5	9.7	37.5	
Control bone	33.2	30.4	1.9	2.75		651.2
	27.7	30.5	1.7	2.30		707.5
	29.1	30.2	2.0	2.44		700.0
Avg	30.0	30.4	1.9	2.50		686.2
Bone Na-loaded	25.3	32.7	1.7	2.41		663.0
	24.4	32.0	1.6	2.25		671.5
	21.2	31.2	.9	2.10		687.0
	21.3	33.0	1.0	2.04		680.0
	21.5	32.0	1.0	2.18		
Avg	22.7	32.2	1.2	2.19		675.0

In the soft tissues, muscle and liver, the basic data also show little change, with the exception of a slight increase in the water and chloride content of the experimental tissues. Potassium and phosphate in muscle both decline, while in liver, both increase. Neither change is striking.

Bone, in contrast to muscle and liver, shows a decrease in water of 7.3 g/100 g of dry fat-free solids, equivalent to a loss of 24% of total bone water. There was an increase of 1.8 meq of sodium/100 g of fat-free dry solids, or 6%. Potassium, chloride, and calcium concentrations fell slightly.

Discussion. Table III presents the extracellular spaces and the intracellular ion concentrations found in muscle and liver. The changes observed in these tissues in response to sodium loading were small and tended to be in opposite directions in the 2 tissues. The ECF of muscle increased 5% in the sodium-loaded rats, while liver ECF decreased 4%. Intracellular muscle potassium concentration decreased slightly, while liver potassium increased an equally small amount. Using the sodium space in liver as a measure of the extracellular fluid, as described, values for intracellular chloride of 15-21 meq/kg of ICW were found. The values were somewhat higher in the experimental animals. This chloride may be distributed uniformly through the liver cells or, more probably, may be present at a higher concentration in the Kupfer cells(7). The exact location cannot be determined from the data presented here. There was no increase in the intracellular sodium of muscle. Actually, it was somewhat lower than in the control animals. Since an assumed value for intracellular sodium was used in liver, based on muscle composition, no data are available as to its actual intracellular concentrations. However, there was no increase in the total amount of liver sodium (Table II). None of the changes observed in these tissues were statistically significant. The small reciprocal shifts in ions observed in liver and muscle are in keeping with the findings of other workers concerning the respective *in vitro* ion requirements of these tissues for optimum carbohydrate metabolism (9,10). Opposite responses of the intracellular electrolytes of liver and muscle have also been described by the authors in acidosis in dogs(1).

TABLE III. Average Intracellular Composition of Muscle and Liver in Control and Sodium-loaded Rats.

	Extracellular space, cc/kg tissue H_2O	Na	K	Cl	P
		mMols/kg intracellular H_2O			
Control musc.	133	7.2	171	(0)*	112
Muscle Na-loaded	139	6.2	166	(0)*	108
p	>.5	>.5	>.5		>.5
Control liver	207	(7.2)†	170	15.3	190
Liver Na-loaded	198	(6.2)†	174	20.3	190
p	>.5	>.5	>.5	>.5	>.5

* Assumed not to penetrate cell membrane.
† Assumed on basis of muscle composition.

TABLE IV. Average Changes in Bone Water in Chronic Sodium Loading.

	Total H_2O	Extracellular H_2O	Intracellular H_2O + H_2O of crystallization
	g/kg bone mineral		
Control	450	324	126
Na-loaded	340	291	50
% change	−24	−10.2	−60
p	<.05	>.1	<.01

TABLE V. Average Changes in Bone Electrolytes in Chronic Na Loading.

	Na	K	Ca
	meq/kg bone mineral*		
Control	408	26.6	10,301
Na-loaded	443	17.6	10,145
% change	+8.6	−34.0	−1.5
p	<.02	>.1	>.1

* Corrected for ECF electrolytes.

In contrast to the constancy of liver and muscle composition following sodium loading, bone exhibited considerable change in both sodium and water content. These changes are presented in Tables IV and V. Values are presented as units/kg bone mineral, and, in the case of the electrolytes, have been corrected for the electrolyte contained in the extracellular fluid. This base of reference was employed because approximately one-third of the total bone solids are organic collagen(11), the water of which is more than 90% extracellular in composition(3). When the electrolytes present in the ECF of bone are subtracted from total bone electrolytes, the organic matrix is virtually electrolyte-free, the remainder of the ions being associated with the crystalline solids. *In vivo,* sodium is probably limited to the surface area of these crystals, or to two-thirds of the total mineral(8), so that its effective concentration in the skeleton is probably higher than the figures presented here.

Table IV presents changes in the water content of bone occurring in response to sodium loading. A significant loss of 24% of total bone water occurred in the experimental animals. There was a small loss of extracellular water, but the major part of the water loss was accounted for by a decrease of 60% in the sum of the intracellular and crystal water of the bone. In the dense cortical bone studied here, cell water probably constitutes only some 5% of total bone water; therefore, it is probable that most of this water was lost from the crystalline phase of bone water, due to rearrangement or loss of the hydration shells of the crystals.

Since the extracellular spaces in the bones of the experimental animals were smaller than in the controls, it is apparent that no sodium was added to bone from this source. Sodium associated with bone mineral showed a rise in concentration from 408 meq in the control bones to 443 meq in the experimental bones, representing an 8.6% increase in bone crystal sodium. Changes in potassium and calcium were not statistically significant.

A reciprocal relationship between water and sodium concentrations in bone mineral has been described by the authors in acute sodium depletion due to mercurial diuretics and also in response to aging(12). The equimolar, heteroionic exchange of sodium for hydronium ions has been demonstrated in bone(8), and such a simple exchange may account for part of the water lost in these animals. Sodium can also substitute for calcium at the surface of the bone crystals, on an equimolar basis (13), and a small, though statistically insignificant, fall in bone calcium was noted in the experimental bones. If such an exchange did occur, it would involve the substitution of a univalent ion for a divalent ion, and, furthermore, an ion whose ionic radius in the hydrated state is smaller than calcium. Both of these factors could effect a reduction of the hydration shell of the crystal, resulting in a decrease in bone water. Whether either or both of these mechanisms plays a role in the changes in sodium and water concentrations reported here is purely speculative.

The relative constancy of the plasma, muscle and liver composition in these animals, despite the massive loads of sodium chloride, is in keeping with the plasma data of Gamble *et al.* in infants given large loads of sodium salts(14) and with the data of the authors on the cellular tissues of dogs and rats during various forms of sodium depletion(1,12). The failure of muscle and liver to show an in-

creased sodium content after sodium loading confirms the impression gained from these previous studies; namely, that the sodium content of extracellular fluid and soft tissue cells is closely guarded in the normal animal and can only be altered by extreme situations. The changes in the sodium content of bone mineral, on the other hand, confirm the concept that this phase of the skeleton acts as a reservoir of sodium which is able, under appropriate circumstances not only to release sodium into the extracellular fluid, but also to store this ion.

Summary. 1) The composition of plasma, muscle, liver and bone was examined in adult male rats. Three rats served as controls, and 5 rats were given a daily load of sodium chloride approximating 50% of the total body sodium for 24 days. 2) The sodium content of plasma, muscle and liver was unchanged in the experimental animals at the end of this time. However, bone mineral sodium increased significantly, and bone water declined.

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Effect of Fasting or Underfeeding on Glucose Tolerance of Rats.* (23625)

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Humans(1) and rabbits(2) fasted for several days show glucose tolerance curves of the diabetic type, *i.e.*, increased elevation and delayed decline of blood sugar levels after glucose administration. The degree of diminution in glucose tolerance is proportionate to the duration of the fast. Hill *et al.*(3) found that in rats fasted for 96 hours (sex, weight and previous diet not specified) which received 400 mg glucose/100 g body wt by stomach tube, blood sugar progressively rose and reached 280 mg% 5 hours after the gavage, whereas it did not exceed 130 mg% if the rats were fed a diet rich in glucose during the 96 hours preceding the tolerance test. We have investigated more extensively the glucose tolerance of rats fasted for 1 to 8 days and rats subjected to chronic undernutrition, which causes a variety of changes in carbohydrate metabolism(4,5,6).

Method. Male Sprague-Dawley rats fed Rockland diet were used. Glucose was given by gavage (300 mg/100 g body wt., 30% solution, or 400 mg/100 g, 40% solution) or into the saphenous vein exposed without anesthesia (100 mg/100 g, 40% solution). Oral glucose tolerance tests were performed in rats fasted for 1, 4, 6 or 8 days and intravenous tolerance tests also in rats which were not fasted. Before fasting these animals were fed

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