Summary. Rhesus moneys were fed cholesterol along with mustard oil (iodine No. 104), coconut oil (iodine No. 9), and sesame oil (iodine No. 110). Fecal cholesterol and plasma cholesterol, phospholipid, lipoproteins and nonesterified fatty acids (NEFA) were estimated at intervals up to 6 months. Supplementation of cholesterol feeding with mustard and coconut oils raised plasma cholesterol and reduced fecal cholesterol to the same degree. The above changes were most marked when the animals were fed sesame oil. A relative decrease in the α-lipoprotein % to β + O lipoproteins was observed in monkeys fed the different oils. Plasma NEFA increased after cholesterol feeding and was highest in animals fed sesame oil. The depressant effect of the different oils on plasma cholesterol level does not seem to be due to saturation, unsaturation or fatty acid composition of the oil.

1. Ahrens, E. H. Jr., *Am. J. Med.,* 1957, v23, 928.
2. Connor, W. E., Hodges, R. E., Bleiler, R. E., *J. Clin. Invest.,* 1961, v40, 894.
3. Swell, L., Flick, D. F., *Am. J. Physiol.,* 1953, v174, 51.
4. Lin, T. M., Karvinen, E., Ivy, A. C., *Am. J. Physiol.,* 1955, v182, 86.
5. ———, *ibid.,* 1956, v187, 173.
6. Aftergood, L., Deuel, H. J., Jr., Alfin-Slater, R. B., *J. Nutrition,* 1957, v62, 129.
7. Portman, O. W., Hegsted, D. M., Stare, F. J., Bruno, D., Murphy, R., Sinisterra, L., *J. Exp. Med.,* 1956, v104, 817.
8. Gordon, H., Lewis, B., Eales, L., Brock, J. F., *Lancet,* 1957, v2, 1299.
9. Jones, R. J., Reiss, O. K., Huffman, S., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 88.
10. Swell, L., Flick, D. F., Field, H. Jr., Treadwell, C. R., *Am. J. Physiol.,* 1955, v180, 124.
11. Keys, A., Anderson, J. T., Grande, F., *Lancet,* 1957, v1, 60.
12. Sobel, A. E., Meyer, A. M., *J. Biol. Chem.,* 1945, v157, 255.
13. Youngberg, G. E., Youngberg, M. V., *J. Lab. Clin. Med.,* 1930, v16, 158.
14. Dole, V. P., Meinertz, H., *J. Biol. Chem.,* 1960, v235, 2595.
15. Banerjee, S., Bhadury, J. N., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 340.
16. Malmros, H., Wigaud, G., *Lancet,* 1957, v2, 1.
17. Horlick, L., Craig, B. M., *ibid.,* 1957, v2, 566.
18. Jencks, W. P., Hyatt, M. R., Jetton, M. R., Mattingly, T. W., Durrum, E. L., *J. Clin. Invest.,* 1956, v35, 980.
19. Dole, V. P., Bierman, E. L., Roberts, T. N., *ibid.,* 1957, v36, 884.
20. Popjak, G., *Biochem. J.,* 1946, v40, 608.
21. Waddell, Wm. R., Geyer, R. P., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 251.
22. Sudborough, J. J., Watson, H. E., Ayyar, P. R., Mascarenhas, U. M., *J. Indian Inst. Sci.,* 1926, vA9, 43.
23. Lepkovsky, S., Feskov, G. V., Evans, H. M., *J. Am. Chem. Soc.,* 1936, v58, 978.
24. Jamieson, G. S., Baughman, W. F., *ibid.,* 1924, v46, 775.
25. *British Pharmacopeia,* Pharmaceutical Press, London, 1953, p. 754.

Received October 2, 1961. P.S.E.B.M., 1962, v109.

Intracellular Sodium Components in Rat Skeletal Muscle.* (27189)

ROSEMARY SUSAT† AND JOHN C. VANATTA
Department of Physiology, University of Texas, Southwestern Medical School, Dallas

Glycerated frog skeletal muscle was found by Fenn(1) to contain sodium after immersion in 50% glycerol for times varying from 12 hr to 4 days. The temperature is not stated. The quantity present was 3.82 to 12.3 meq/kg wet weight. The amount of sodium did not appear to be related to the time of immersion in glycerol. More recently Craig(2) found that the sodium content of frog skeletal muscle decreased steadily after being placed in glycerol, from time zero when sodium value was 27.4 ± 0.3‡ meq/kg, to

* This investigation was supported in part by PHS research grant from Nat. Heart Inst., P.H.S.

† This investigation was carried out during the tenure of a Postdoctoral Fellowship from Division of General Medical Sciences, U.S.P.H.S.

‡ Standard deviation of mean.

1.8 ± 0.1 meq/kg at 48 hours and to 0.2 meq/kg at 90 days. Temperature was 4°C.

In our laboratory the sodium content of rat skeletal muscle glycerated for 2 days at −20°C was 5.26 ± 0.35 meq/kg. This report is a study of the sodium present in glycerated rat skeletal muscle, which remains after further extraction with 0.08 M KCl. Experiments were designed to obtain information concerning the possible location of this intracellular sodium.

Methods. Thigh skeletal muscle, free of gross fat and tendon, from a Sprague-Dawley rat was shredded, weighed and placed in a 80 ml test tube containing 50% glycerol in de-ionized water at −20°C. The time of extraction varied from 1 to 6 days. The glycerol was changed once during this period. At the end of the extraction period the glycerol was decanted. The muscle was then subjected to extraction in one of the following solvent systems: 1. in 0.08 M KCl; 2. in 0.08 M KCl followed by extraction in de-ionized water; or, 3. in de-ionized water. All of these extractions were at 4°C. Unless otherwise indicated, the standard extraction with 0.08 M KCl was for a total of 5 hr with the bath being changed at approximately 2.5 hr. Variations in time of extraction, and of number of times fresh bath was placed on the muscle are indicated in Table I. The osmolar concentration of the bath was measured by freezing point depression.

When the extraction was completed the muscle was dry ashed. The extracted muscle was transferred to a Vycor crucible, covered with 4 N H_2SO_4, digested on a steam bath for 2 hr and ashed in a muffle furnace at 550°C for 8 hr. The ash was dissolved and analyzed for sodium content on the Beckman flame photometer.

The rate of exchange of this sodium component with Na^{24} in a 0.02 M NaCl bath was measured. Muscle, glycerated for 1 or 2 days, was immersed from 10 min. to 3 hr in 0.02 M NaCl containing approximately 10 μc/meq. At the end of immersion period the muscle was placed in 0.08 M KCl for 5 hr. This bath was not changed. Aliquots were taken of the bath at the end of this time for determination of the Na^{24} content. The muscle was blotted on filter paper, transferred to a tared Vycor crucible, and then reweighed. It was dry ashed, and the ash dissolved in water. The Na^{24} and Na^{23} determinations were carried out on this solution. All determinations of Na^{24} were made on a Packard Tri-Carb β-ray scintillation counter. Na^{24} concentrations were corrected for decay.

Na^{24} content of the muscle sample was corrected for the Na^{24} present in solution in the KCl by the following calculations: Original wet weight of sample × 0.30 = estimated dry weight of muscle. Weight of sample after KCl extraction − estimated dry weight = weight of KCl bath in sample. Weight of KCl bath × Sp. Gr. × Conc. Na^{24} in bath = Na^{24} in KCl bath in sample. Specific gravity was assumed to be 1.000. Because of the difficulty of determining small amounts of Na^{23} in presence of high concentration of KCl, similar corrections were not made for Na^{23} content.

The ratio of specific activity of the sodium in the muscle to that in the bath was calculated.

For analysis of the ether soluble compounds in muscle after glycerol and KCl extractions, the muscle was homogenized and extracted with hot ethanol, 1:1 diethyl ether-ethanol, and diethyl ether in that order. The solvents were filtered, pooled and evaporated to dryness. The residue was then reextracted with diethyl ether and insoluble material discarded. The ether was again dried and the residue extracted with acetone which was discarded. The residue, which remained after the acetone extraction was dissolved in petroleum ether and this solution washed 2 times with 70% alcohol. The 70% alcohol washings were pooled, and analysis for Na content carried out on a Model B Beckman flame photometer using standard Na solutions prepared in 70% alcohol for reference.

Electron micrographs were obtained of the glycerated muscle after extraction in KCl and also after further extraction with water. After treatment, small blocks of tissue were fixed in a chrome-osmium solution(3), dehydrated in alcohols and embedded in methacrylate. Ultrathin sections were obtained from these blocks for electron microscopy.

TABLE I. Sodium Content of Glycerated Muscle Samples after Extraction with 0.08 M KCl and/or De-ionized Water.

Date	No. of samples	Time in glycerol, days	Time in KCl, hr	No. of KCl baths	Time in water, hr	No. of water baths	Na content, meq/kg	Osmolarity of final bath, mosm/l
11/28/60	8	2	5	2	16	2	.00*	
2/13/61	4	2	5	1			.24	754
				2			.44	245
				3			.80	172
				4			.70	153
2/28/61	3	2	0	0	16–18	4	.39†	
3/13/61	4	2	0	0	5	1	3.22	463
						2	1.24	69
						3	1.43	41
						4	1.39	4
5/15/61	4	2	5	2	2	1	.72	21
						2	.67	2.8
						3	1.07	1.8
						4	1.38	.0
3/24/61	1	5	5	2	16	4	.53	
5/15/61	2	7	5	2	16–18	4	.71†	
3/24/61	1	10	5	2	16–18	4	.90	
3/15/61	3	14	5	2	16–18	4	.29†	
5/15/61	1	14	5	2	20	4	.28‡	
11/ 1/60	10	21	5	2	16–18	2	.00§	
4/21/61	1	21	18	1	3	3	2.90	

* No Na found in any sample. † Mean. ‡ Electron micrograph made of companion muscle similarly treated. § See text.

Results and discussion. 1. *Effects of time of glycerol extraction.* Studies to determine the effect of various extraction times in 50% glycerol-water on sodium content of muscle were carried out. In each case the muscle was further extracted using the standard KCl extraction described. The sodium content of 6 samples extracted 24 hr averaged 1.82 meq/kg wet weight, of 7 samples extracted 48 hr averaged 1.68, of 10 samples extracted 120 hr averaged 1.24 and of 2 samples extracted 144 hr averaged 1.87 meq/kg. This was interpreted as indicating no effect of increasing the time of extraction in 50% glycerol-water on the Na content of the muscle.

2. *Effect of osmotic pressure.* Table I shows the effect of hypotonic solutions upon sodium content. Seventeen out of 18 samples had no sodium in them after 5 hours in KCl followed by 16 hours in water and the remaining sample contained 0.17 meq/kg. These were the results of experiments done on 11-1-60 and 11-28-60. Attempts to repeat these experiments were unsuccessful, and sodium content of 16 samples extracted in 3 to 4 water baths with or without prior KCl immersion was from 0.26 to 2.90 meq/kg. The cause of these differences is not apparent.

The results on the initial 17 samples which had no sodium in them after extraction with water suggested that the sodium present after KCl extraction might be held in some structure by a membrane such as in the nuclei or mitochondria. Such structures might act as osmometers, and when placed in a hypotonic solution would swell and perhaps become disrupted, releasing the sodium contained within.

Since 50% glycerol-water has a calculated osmolarity of 5,435 mosm/1 there is enough glycerol remaining in and on the muscle significantly to raise the expected osmolarity of the bath. In the experiments of 3-13-61 it can be seen that 3 washings following glycerol are necessary to give a tonicity of less than 50 mosm/1, and that a fourth washing assures a bath with less than 5 mosm/l.

It was found in experiments of 2-13-61, 3-13-61 and 5-16-61, that there was no relation between sodium content of the muscle and

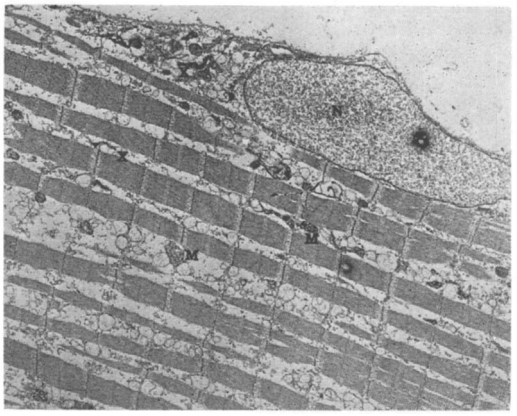

FIG. 1. An electron micrograph of muscle after extraction in 50% glycerol-water, then 0.08 M KCl, then de-ionized water. N is nucleus; M, intact mitochondria; and X, collapsed mitochondria. Over 90% of mitochondria were collapsed when viewed electron-microscopically (3417×).

osmolarity of the bath, nor was there critical osmolar concentration below which all sodium could be removed from the muscle.

3. *Electron micrographs.* The electron micrographs of glycerated KCl extracted muscle showed that the nuclei were anatomically intact. There was no disruption of nuclear membrane, but 90% of the mitochondria were collapsed. Electron micrographs of such muscle further extracted in hypotonic water baths showed no change in the condition of the nuclei or mitochondria. A typical micrograph is shown in Fig. 1. These results are interpreted as indicating that the nuclei and/or mitochondria might be the site of the sodium present, but that these structures do not behave as osmometers.

4. *Connective tissue binding.* There is conflicting evidence in the literature regarding the question of Na of rat muscle being bound to connective tissue. Audia(4) concludes that there is no Na in tendon in excess of water and hence no binding to connective tissue matrix. Levitt(5) and co-workers conclude after similar anlyses that there is binding of Na by connective tissue matrix. Calculating from their data, there is 6.6 meq bound Na/kg tendon wet weight. Muntwyler(6) analyzed for connective tissue Na in the muscle of the dog and calculated that there was 187 g of connective tissue wet weight per kg of muscle wet weight. If this were true for the rat, then one might expect the Na bound to connective tissue to be 1.2 meq/kg of muscle.

Since this calculated value is so near the value observed in glycerated, KCl extracted muscle, it suggested that connective tissue might be the site of the bound Na in glycerated, KCl extracted muscle.

Manery(7) discusses the evidence that connective tissue in muscle is the same as connective tissue of tendon. On the basis of this evidence one would expect the connective tissue to be in higher concentration in the ends of a muscle than in the middle. Harris(8) found that a small fraction of frog muscle Na does not exchange with tracer Na, that this fraction resists water extraction and is associated with the ends of the muscle. He concludes that this Na is held in the connective tissue of muscle.

To determine if the Na fraction present in glycerated, KCl extracted rat muscle was bound by connective tissue, muscle was divided into its terminal fourths, and middle half, and then extracted. Four groups of muscles were so divided and extracted. Average Na content of the terminal fourths was 2.02 meq/kg and of the middle half was 2.78 meq/kg. The difference between these figures is neither of the right direction to indicate connective tissue binding of the sodium, nor is it statistically significant.

5. *Ether soluble sodium.* The possibility existed that the sodium might be present in ether soluble compounds. The sodium in such compounds in 10 samples of glycerol, KCl extracted muscle was 0.28 ± 0.06 meq/kg.

6. *Dynamics of sodium exchange.* In the Na^{24} experiments the magnitude of the correction of total Na^{24} in the muscle for the Na^{24} which remains in the KCl bath which penetrates the muscle is of interest. Na^{24} in the bath as calculated averaged 6.5% of total Na^{24} in the samples incubated 1 and 3 hr. In those incubated less time, with consequent less Na^{24} total in the sample, the percentage in the bath was of course much higher, and in one 10 min. sample the bath appeared to account for all radioactivity present.

The results of the exchange experiments are given in Fig. 2. The data are interpreted

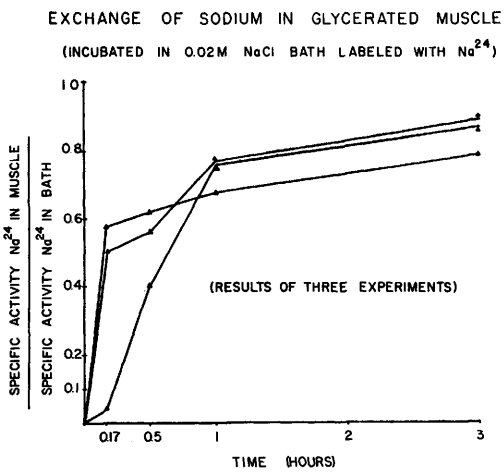

FIG. 2. Results of studies of Na^{24} exchange with sodium in glycerated muscle. All points are avg of duplicate determinations.

as indicating that there are probably at least 2 compartments in the system, a rapidly exchanging and a slowly exchanging compartment.

Conclusions. 1. Rat skeletal muscle is found to contain sodium after extraction with 50% glycerol-water followed by 0.08 M KCl. There was 1.24 meq/kg wet weight in a typical series. Sodium was present after further extraction with distilled water. Average Na content of 16 samples so extracted was 0.84 meq/kg. 2. Electronmicroscopic studies reveal intact nuclei and collapsed mitochondria as possible sites of this sodium. 3. The sodium present after glycerine and KCl extraction exchanges with Na^{24}. The rate is not a simple first order reaction, and suggests that the sodium exists in at least two compartments. 4. A small portion of the sodium appears to be in a compound which is soluble in ether. 5. Evidence is presented that this sodium is not associated with the matrix of the connective tissue in muscle.

Summary. The premise that intracellular sodium is functionally a simple solution in a cell membrane is questioned by the authors and others(2,9). The data reported are interpreted as indicating that there is a component of intracellular sodium which is characterized by not being exchangeable with potassium ions. Fenn felt that such sodium ions were bound to myosin(1). The nuclei and mitochondria are suggested as additional possible sites of binding of such sodium.

The authors wish to thank Dr. Charles Ashworth, Dept. of Pathology, Univ. of Texas, Southwestern Medical School, for aiding in interpretation of the electron micrographs.

1. Fenn, W. O., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 783.
2. Craig, A. B., Jr., Ohr, E. A., Fenn, W. O., *Am. J. Physiol.*, 1961, v200, 561.
3. Dalton, A. J., *Anat. Rec.*, 1955, v121, 281.
4. Audia, M., *Am. J. Physiol.*, 1958, v195, 702.
5. Levitt, M. F., Turner, L. B., Sweet, A. Y., Pandiri, D., *J. Clin. Invest.*, 1956, v35, 98.
6. Muntwyler, E., Mellors, R. C., Mautz, F. R., Mangun, G. H., *J. Biol. Chem.*, 1940, v134, 389.
7. Manery, J. F., Danielson, I. S., Hastings, A. B., *ibid.*, 1938, v124, 359.
8. Harris, E. J., Steinbach, H. B., *J. Physiol.*, 1956, v133, 385.
9. Conway, E. J., *Physiol. Rev.*, 1957, v84, 37.

Received October 4, 1961. P.S.E.B.M., 1962, v109.

Latex Agglutination Reactions Between Human Chorionic Gonadotropin and Rabbit Antibody.* (27190)

J. L. ROBBINS, G. A. HILL, B. N. CARLE, J. H. CARLQUIST, AND S. MARCUS
Latter Day Saints Hospital and University of Utah, Dept. of Microbiology, College of Medicine, Salt Lake City

The antigenicity of the partially purified protein hormone, human chorionic gonadotropin (HCG), in the rabbit has been reported(1). This rabbit antibody has been employed in precipitin and in hemagglutination-inhibition technics as a serologic test for pregnancy(2,3).

* Supported by grants from the Latter Day Saints Hospital Research Foundation and Nat. Inst. Health, Division of General Medical Sciences.