tain other tissues or cells (polymorphonuclear cells or liver), there was a report of Levey and Medawar (7) that L cells and mouse epidermal cells did contain some of the antigens since antisera directed against them had immunopressive properties, and were capable of prolonging skin homografts. These authors concluded that the antigens are not unique for lymphocytic cells.

Our results, like the results of other investigators (8,9) show that injection of normal rabbit serum caused a certain degree of immunosuppression. The nature of the suppression remains to be investigated.

Summary. Rabbit antisera directed against cultured mouse lymphocytic leukemia L1210 cells as well as those directed against the ascitis form were shown to be immunosuppressive when injected into mice prior to immunization with sheep erythrocytes. The extent of immunosuppression was comparable to the one induced by injection of antisera directed against mouse spleen and lymph node antigens. The degree of immunosupression was quantitated by assaying the number of antibody forming cells by plaque formation in a sheep erythrocyte medium, and by titration of humoral hemagglutinins.

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Intracellular Distribution of Calcium-45 in Arteries of Normal and Hypertensive Dogs* (32742)

Henry W. Overbeck[1] and Loyal L. Conrad (Introduced by J. B. Scott)

Veterans Administration Hospital, Oklahoma City, Oklahoma; and the Departments of Medicine and Physiology, University of Oklahoma Medical Center, Oklahoma City, Oklahoma 73104.

Evidence has accumulated suggesting that the calcium ion plays an important role in maintenance of the blood pressure level in man both in normotension and in some forms of secondary hypertension. This evidence has been reviewed recently by several authors (1, 2). In addition, well documented changes occur in the electrolyte and water composition of walls of small and large arteries in hypertension (3). These changes include increases in calcium content of small arteries (4) and of arterioles (1) in experimental hypertension, and suggest that alterations in arterial wall calcium metabolism may accompany or underlie hypertension. In view of these relationships and findings, it is of interest to study the intracellular distribution of calcium of arterial wall in normotension and hypertension. The technique of differential centrifugation is appropriate for such an investigation.

Although the intracellular distribution of ^{45}Ca in cardiac muscle has been reported (5,6), there are no published simliar studies of vascular wall tissue. In the present study radial arterial, carotid arterial, and descending

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[1] Present address: Dept. of Physiology, Michigan State Univ., E. Lansing, Michigan 48823.

thoracic aortic tissues were differentially centrifuged, and the intracellular distribution of ^{45}Ca was measured both in normal dogs and in dogs with chronic experimental renal hypertension.

Methods. Chronic renal hypertension was created in 12 healthy male mongrel dogs weighing 40-60 lbs. (hypertensive group) by performing unilateral nephrectomy and wrapping the remaining kidney in cellophane according to the method of Page (7). This hypertension was documented by weekly femoral arterial punctures and persisted at least 4 weeks before tissue was obtained for analysis. An additional seven similar dogs (control group) underwent a sham operation consisting of unilateral nephrectomy and dissection of the remaining kidney free of the fat pad. None of these 7 dogs became hypertensive over periods of 4–12 weeks postoperatively. In both the hypertensive group and the control group, procaine penicillin 100,000 units and streptomycin 0.1 gm were administered intramuscularly daily for the first 5 postoperative days. Postoperatively, the good general health of the animals was verified by observation, determination of serum sodium, potassium, and calcium concentrations, hematocrit, blood urea nitrogen, and by urinalyses. In a third group of 10 similar dogs (normotensive group), no operative procedure was done prior to obtaining arterial tissue. Normotension was verified by repeated femoral arterial punctures over the course of 3 to 4 weeks, and then, after loading with ^{45}Ca, arterial tissue was obtained. All three groups were maintained on a balanced diet of standard dog chow.

At the time of sacrifice, the dogs were anesthetized with sodium pentobarbital, 35 mg/kg, and given 10,000 USP units of heparin sodium intravenously. A loading dose of 200–400 μC of calcium-45 was given intravenously approximately 2 hours before tissue was obtained. Three-inch specimens of carotid artery, and in some cases, radial artery, and descending thoracic aorta were quickly dissected and removed, freed of adventitia and blood as well as possible, and placed in 0.25 *M* sucrose solution at about 0°C prior to homogenization.

Cell homogenization and fractionation were carried out according to the method described in detail by Schneider (8). Centrifugation was performed in a Spinco model L preparatory centrifuge equipped with an SW 39L or 30.2 rotor. An additional fraction was obtained by centrifugation at 123,000*g* for 17 hours. After final washing, pellets were dried at 50°C for 24 hours and weighed. Radioactvity was determined in each fraction using standard gas-flow counting procedures on a Tracerlab Omniguard low beta-counter. Values for each fraction were expressed as net counts per minute per gram dry weight of the fraction and also as percentage of the summed counts per minute per gram of all fractions obtained from the arterial specimen. The dosage of isotope differed in each animal, as did body weight, volume of distribution, and time of sacrifice. The pellet obtained by centrifugation at 700*g* was sectioned and examined by light microscopy to identify its composition. It was found to be composed mainly of intact nuclei. Adequate methods for verifying the composition of the other pellets were not available, and therefore all fractions are identified by the *g* force required for separation. Serum calcium determinations on venous blood were made according to the method of Zak, *et al.* (9). Serum potassium and sodium concentrations were determined on a Baird Atomic flame Photometer, model KY, and serum blood urea nitrogen was determined on a Technicon AutoAnalyzer.

In order to test for statistical similarity of calcium distribution in carotid artery fractions of the three groups of dogs studied, simultaneous 95% confidence intervals for the differences between the means were calculated by Sheffé's method after Bartlett's test was used to verify homogeneity of variances. Homogeneity of variances is required for application of Sheffé's test (10).

Results. Clinical observations on the 25 dogs sampled are presented in Table I. The hypertensive, control, and normotensive groups were well matched for weight and preoperative blood pressure. Mean rise in blood pressure in the hypertensive group was 41 mm Hg, with mean duration of hypertension 7.6 weeks. Mean change in blood pressure in the control group was —2 mm Hg. The number of weeks between surgical pro-

TABLE I. Clinical Observations.[a]

	Hypertensive group	Control group	Normotensive group
1. Weeks between surgical procedures	9.9 (12)	9.7 (7)	— —
2. Mean preoperative BP (mm Hg)	128 (12)	125 (7)	126 (10)
3. Mean postoperative BP (mm Hg)	169 (12)	123 (7)	— —
4. Weeks duration hypertension	7.6 (12)	— —	— —
5. Mean preoperative body wt. (lbs)	49 (12)	46 (7)	51 (10)
6. Mean postoperative body wt. (lbs)	50 (12)	48 (7)	— —
7. Mean preoperative HCT (%)	42.1 (9)	46.1 (6)	42.6 (6)
8. Mean postoperative HCT (%)	41.3 (9)	41.6 (6)	— —
9. Mean preoperative serum [Na^+] (meq/liter)	152 (9)	153 (6)	152 (6)
10. Mean postoperative serum [Na^+] (meq/liter)	153 (9)	150 (6)	— —
11. Mean preoperative serum [K^+] (meq/liter)	4.4 (9)	4.0 (6)	4.4 (6)
12. Mean postoperative serum [K^+] (meq/liter)	4.3 (9)	4.1 (6)	— —
13. Mean postoperative serum [Ca^{++}] (meq/liter)	5.1 (5)	5.1 (5)	5.0 (1)
14. Mean preoperative BUN (mg/100 ml)	22 (10)	22 (5)	20 (10)
15. Mean postoperative BUN (mg/100 ml)	27 (10)	26 (5)	— —
16. Final urinalysis (mean albuminuria)	2+ (11)	1+ (5)	— —
17. Final urinalysis (mean sp gr)	1.029 (11)	1.041 (5)	— —

[a] Numbers in parentheses = n.

cedures was nearly identical in the 2 groups. There were no significant abnormalities in measured blood chemistries or urinary functions at time of sacrifice in any group. The general health of all groups was good at time of sacrifice.

The intracellular distribution of ^{45}Ca in artery walls of the 3 groups is presented in Table II. Mean weights of carotid artery cell fractions obtained from animals in the 3 groups were not significantly different. More aortic and less radial arterial tissue was obtained and therefore mean weights of aortic and radial artery fractions were significantly greater and lesser, respectively, than carotid artery fractions.

The simultaneous confidence intervals for the differences between means for carotid artery fractions are presented in Table III. Since all intervals include zero, therefore the null hypothesis of equality of means is accepted at the 95% level of significance. This is statistical evidence that intracellular distribution of calcium is similar in carotid artery fractions of the normotensive, control, and hypertensive dogs.

Discussion. The present project represents a new approach to the study of vascular wall calcium metabolism in normal and hypertensive animals. Since there is every reason to believe that the vasoactive actions of the calcium ion occur primarily within the vascular smooth muscle cell, a study primarily directed at the intracellular composition of arterial wall is perhaps potentially more informative than gross chemical analysis of arterial wall. Indeed, increases in total calcium content of vascular wall in hypertension might merely indicate extracellular calcium deposits of accelerated arteriosclerosis accompanying hypertension. It has not been documented that the increased calcium content reported in arterial wall in experimental hypertension is intracellular in location (1,4). Furthermore, intracellular calcium distribution in vascular smooth muscle might well be abnormal in hypertension, even if vascular wall content were normal. It follows from these considerations that differential centrifugation is potentially a valuable technique for study of vascular wall ion metabolism.

The noncellular elastin and collagen composition of artery walls differs significantly at various sites along the arterial tree. However most elastin and collagen fibers are discarded during homogenization in preparation for differential centrifugation. A very small portion of elastin and collagen may appear

TABLE II. Differential Centrifugation of Artery Wall; Intracellular Distribution of ^{45}Ca (Mean ± SEM).

700*g*		5000*g*		123,000*g*		17-hour fraction		Supernatant
(cpm/gm)	(%)	(cpm/gm)	(%)	(cpm/gm)	(%)	(cpm/gm)	(%)	(cpm/ml)
			Carotid artery, normotensive group (n=10)					
367	26.9 ±2.3	270	20.1 ±1.8	459	34.3 ±1.8	243	18.6 ±1.2	5.8
			Carotid artery, control group (n=7)					
330	25.4 ±2.1	261	19.8 ±2.1	460	34.9 ±2.5	271	20.0 ±2.4	9.2
			Carotid artery, hypertensive group (n=12)					
540	27.8 ±3.6	450	20.7 ±2.0	728	31.6 ±2.4	503	19.9 ±2.7	10.4
			Thoracic aorta, control group (n=4)					
1738	16.4 ±1.4	2040	19.2 ±2.5	4300	39.0 ±1.2	2812	25.3 ±2.9	56.9
			Thoracic aorta, hypertensive group (n=3)					
1975	24.5 ±4.8	1483	17.9 ±0.8	2435	28.5 ±3.9	2472	29.1 ±2.4	60.4
			Radial artery, control group (n=2)					
76	15.9 ±8.4	74	16.0 ±2.5	211	47.8 ±14.0	93	20.2 ±3.3	2.4
			Radial artery, hypertensive group (n=10)					
301	23.8 ±4.3	334	26.8 ±4.9	293	25.6 ±4.1	306	24.9 ±7.0	8.4

as debris in the 700*g* fraction but not in the 5000*g*, 123,000*g*, or 17-hour fractions. Therefore the techniques used in this study essentially measure only intracellular components of the vascular wall. The cells of all arterial walls are predominantly smooth muscle; endothelial cells and connective tissue cells make up only a small portion of the cellular composition (11). Thus, the present study has probably measured mainly the distribution of ^{45}Ca within the vascular smooth muscle cell. There is the possibility, however, that the calcium distribution may have been significantly altered by the homogenization process.

Of the cellular components studied in carotid artery, the 123,000*g* fraction contained the greatest concentration of ^{45}calcium and probably therefore also the greatest concentration of normally existing calcium. The 5000*g*, 700*g*, and the 17-hour fractions contained decreasing concentrations of calcium

TABLE III. Simultaneous 95% Confidence Intervals for Differences between Means of Carotid Artery Fractions.

Fraction	Intervals (%)
Normotensive group minus hypertensive group	
700*g*	+0.9 ± 14.9
5000*g*	+0.1 ± 14.9
123,000*g*	−2.9 ± 14.9
17-hour	+1.3 ± 14.9
Normotensive group minus control group	
700*g*	+1.5 ± 17.1
5000*g*	+0.3 ± 17.1
123,000*g*	−0.6 ± 17.1
17-hour	−1.4 ± 17.1
Normotensive plus control groups minus hypertensive group	
700*g*	+1.5 ± 13.1
5000*g*	+0.7 ± 13.1
123,000*g*	−2.9 ± 13.1
17-hour	+0.7 ± 13.1

in that order. There has been presented reasonable evidence that the 700*g* fraction represents nuclei. The 5000*g* and the 123,000*g* fractions have not been identified in the present study; however, they usually consist of mitochondria and microsomes, respectively.

Experimental variation was considerably greater in the case of the aorta, possibly due to the presence of calcium in arteriosclerotic deposits that were grossly obvious and could not always be avoided in sampling. Similar deposits were not seen in the carotid and radial arteries. Results were least clear in the case of the radial artery, where experimental variation was highest, possibly due to the small amounts of tissue obtainable. This great variation suggests that within the present limitations of the technique similar analysis of arteriolar tissue would be unrewarding. However, the intracellular distribution of ^{45}Ca appeared to be grossly similar in normal carotid artery, radial artery, and aorta.

Data in the present study may be compared to similar studies by Conrad and Baxter (5,6) of the intracellular distribution of ^{45}Ca in rat myocardium. At 2 hours after a subcutaneous loading dose of ^{45}Ca they found the mean percentage uptakes to be: 700*g* fraction, 22.6%; 5000*g* fraction, 25.1%; 123,000*g* fraction, 36.9%; and 17-hour fraction, 15.5%. Their values were very similar to those obtained in the present study.

In several animals two arteries were sampled simultaneously. Therefore, it was possible to compare the concentration of ^{45}Ca in the cell fractions of the two arteries. The comparison indicated that the concentration of ^{45}Ca in summed carotid arterial fractions was only 7–15% of that in the summed aortic fractions. Similarly, concentration of ^{45}Ca in summed radial arterial fractions was 16–56% of that in the summed carotid arterial fractions. These findings suggest that the calcium content of vascular smooth muscle decreases progressively from the aorta to the radial artery and are similar to the findings of Jones *et al.* (12) that total water and potassium contents decrease progressively from the ascending aorta to the femoral and carotid sites. The data in the present study do not allow calculation of absolute content of calcium in arterial wall.

Within the limitations of the techniques available, the present study helps to characterize the intracellular distribution of the calcium ion in the walls of larger arteries of normal dogs. The present study also indicates that no abnormality in the intracellular distribution of calcium exists in carotid artery wall of the renal hypertensive dog. Thus the present study does not provide evidence that a derangement in intracellular calcium metabolism accompanies or underlies hypertension.

Summary. The intracellular distribution of ^{45}Ca in arterial wall from normal and from experimentally hypertensive male mongrel dogs was studied, because indirect evidence suggests that the calcium ion has an important role in the maintenance of blood pressure, and because well-documented changes occur in the electrolyte and water composition of walls of arteries in hypertension. Chronic renal hypertension was created in 12 dogs by cellophane perinephritis. Arterial tissue was obtained under sodium pentobarbital anesthesia 2 hours after intravenous loading with ^{45}Ca. Tissue was obtained similarly from 7 sham operated control dogs and from 10 normal unoperated dogs. Arterial tissue was differentially centrifuged and the radioactivity of intracellular fractions was determined. Intracellular distribution in aorta and radial artery was similar to that in carotid artery. Mean percentage uptakes by the individual intracellular fractions in normal dog carotid artery were: 700*g* fraction, 26.9%; 5000*g* fraction, 20.1%; 123,000*g* fraction, 34.3%; 17-hour fraction, 18.6%. The intracellular distribution of ^{45}Ca was statistically similar in carotid artery in normal, control, and hypertensive dogs.

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Removal by Heparin–$MnCl_2$ of Nonspecific Rubella Hemagglutinin Serum Inhibitor* (32743)

HARRY A. FELDMAN

Department of Preventive Medicine, State University of New York Upstate Medical Center, Syracuse, New York 13210

The serology of rubella virus infections was simplified, enormously, by the description of methods for demonstrating viral hemagglutination (HA) and antibodies by hemagglutination-inhibition (HI) (1). The hemagglutinin is produced in BHK-21 cells and agglutinates erythrocytes from day-old chicks. The HI tests require that a nonspecific inhibitor of rubella virus hemagglutinin be removed from serum by absorption with kaolin. This report describes another, more effective, system for removing the interfering inhibitor. Antibody titers determined with the new system are compared with those of the kaolin-absorbed sera of 100 elderly persons.

Materials and Methods. Antigen. High titered seed virus (M-33 strain, originally supplied by Dr. Paul Parkman) maintained in RK-13 cells was inoculated into BHK-21 cells (provided by Mr. Monroe Vincent, Microbiological Associates, Inc.) which were fed with Eagle's basal medium without serum. The BHK supernate was tested for HA daily and harvested when the titer was satisfactory, generally 1:16-1:32. Following its harvest, the supernate was treated with ether and Tween 80 (2) and stored frozen until used.[1]

Reference test. This test was performed as described by Stewart, *et al.* (1) except that room temperature incubation was used throughout. Erythrocytes were obtained from pools of day-old chick blood mixed with an equal volume of modified Alsever's solution.[2] Dextrose–gelatin–barbital buffered saline (DGV) was the diluent employed. All sera were inactivated for 30 min at 56°C and absorbed with 25% kaolin for 20 min at room temperature. Before centrifugation, a drop of 25–50% chick erythrocytes was added. After 1 hour at 4°C, the tubes were centrifuged and their supernate removed. These were assumed to represent 1:4 dilutions of the sera.

Heparin–manganous chloride treatment. Aliquots of each serum were treated with heparin-$MnCl_2$ as suggested by Mann *et al.* (4) except that the two reagents were mixed

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[1] Antigen was prepared by Dr. Alvin Novack, formerly of this Department.

[2] Among other erythrocytes tested for reactivity with rubella virus hemagglutinin, it was subsequently found that cells from Muscovy ducks yield titers at least as good as those obtained with day-old chick cells. Adult duck cells are employed routinely in this laboratory.