not evident for several hours. Examination of the paws of the 2 dead animals in this group showed evidence of edema. Thus, it seems likely that the anaphylactoid purpura reaction occurred in all the animals treated with agar and dextran. The complex interactions related to injections of agar and dextran have been indicated particularly in the review by Osler *et al*(17), which emphasizes the role of histamine release, production of anaphylatoxin and the reduction of complement.

Summary. Although both dextran and starch are glucose polymers, the structural dissimilarities of their linkages and chain branchings have the effect of conferring sharp contrast when compared in terms of the characteristic anaphylactoid reaction or the anaphylactoid purpuric reaction in rats. With dextran there was a high incidence of reactions, whereas, with hydroxyethyl starch there were none. Mice exhibited no reactions of this sort with either of these colloids.

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Ca and Ouabain Interaction on Vascular Smooth Muscle.* (30757)

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It has been known for some time that the cardiac glycosides can produce contraction of arterial smooth muscle *in vitro*(1,2). In recent years, evidence has accumulated which indicates also that in therapeutic doses, the glycosides produce constriction of arteriolar and venous beds *in vivo* in dogs(3) and humans(4). The mechanism of action is unknown, although it would appear that it is a direct effect of the glycosides on the smooth muscle which is not mediated through the adrenergic nervous system(3). Since there is now evidence suggesting that the cardiac glycosides produce their effect on cardiac muscle by altering calcium (Ca) metabolism(5-8) and that Ca is intimately involved in contraction of vascular smooth muscle(9,10) these experiments were undertaken to determine the possible role of Ca in the action of ouabain on vascular tissue.

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Methods. Aortas isolated from rabbits weighing between 1 and 2 kg were used. The

rabbits were stunned by a blow on the head. The carotids were cut and the chest opened. The thoracic aorta was removed and placed in Ringer's solution. Spiral strips were prepared from the thoracic aorta. The aorta from each rabbit was cut transversely and one half used as a control for the other half (approximately 50% of the time, the cephalic half was used as control). The strips were tied at both ends and suspended in a muscle warmer containing Ringer's solution of the following composition: NaCl 154 mM, KCl 5.4 mM, $NaHCO_3$ 6 mM, dextrose 11 mM, and various $CaCl_2$ concentrations.

A tension of 1½ g was initially applied to the strips and isometric contractions were recorded by means of a strain gauge (Grass FT-03) on a Grass polygraph. Strips were allowed to equilibrate for 2 hours in low Ca solutions (0.3 mM) or for 2 hours and 15 minutes in normal Ca (2.4 mM) and Ca-free solutions, respectively, before the Ca^{45} and Ca content experiments were started. All solutions were aerated with 95% O, 5% CO_2 (pH 6.9) and maintained at 37°C.

The Ca^{45} uptake was measured by incubating the aortas in radioactive Ringer's solution with varying concentrations of $CaCl_2$ for 30 or 60 minutes with and without ouabain, followed by a 5-minute washout period in identical nonradioactive media. The aortas were then patted dry, weighed, homogenized in 6.2% trichloracetic acid (TCA) (1 ml per 40 mg tissue), centrifuged and total radioactivity in aliquot of the supernatant determined. Ca^{45} was obtained from Oak Ridge National Laboratories as the chloride.

Tissue Ca concentrations were determined under identical conditions in nonradioactive media by the direct micro method using the flame photometer with photo multiplier attachment(11). After the experiments, the tissues were homogenized in 18 volumes of 6.2% TCA and after 30 minutes, centrifuged for 10 minutes at 10,000 g's. The supernatant fluid was diluted 5-fold with an organic diluting fluid as described by Geyer and Bowie and the Ca concentration determined(11). The specific activity of the tissue (SA_t) and media (SA_m) and percentage exchange were determined as follows:

$$SA_t = \frac{Ca^{45}\ (cpm)/g\ tissue}{Ca\ (meq)/g\ tissue}$$

$$SA_m = \frac{Ca^{45}\ (cpm)/ml\ solution}{Ca\ (meq)/ml\ solution}$$

$$\%\ Exchange = \frac{SA_t}{SA_m} \times 100$$

Results. Fig. 1 shows a tracing of a typical contracture induced by ouabain (10^{-4} M) in low Ca solution. Note that the contracture begins in 5 minutes and reaches a near-maximum in approximately 30 minutes. However, in Ca-free solution, no contracture appears in aortas incubated in solution containing ouabain until Ca is added, at which time there is a rapid rise to the near-maximum. The tension again falls to the base line when Ca is subsequently removed. Fig. 2 shows the effects of varying the extracellular Ca concentration $(Ca)_o$ on the ouabain induced contracture. In these experiments, the aortas were preincubated in solution containing 2.4 mM Ca for 2 hours. They were then incubated with ouabain (10^{-5} M) in Ca-normal or Ca-free solutions, washed in Ca-free solution without ouabain and then a small amount of Ca added to determine if contracture developed. (The particular Ca concentration of 0.3 mM was used since it had previously been determined that this was the approximate $(Ca)_o$ associated with an optimum Ca influx in potassium contracture(10).) In Fig. 2A, it can be seen that varying the $(Ca)_o$ from Ca-free to low Ca (0.3 mM) has no effect on tension in aortas which have been preincubated in 2.4 mM Ca solution for 2 hours. Furthermore, the addition of ouabain (10^{-5} M) in Ringer's solution containing the 2.4 mM Ca had no effect in the first 20 minutes and no appreciable effect following a washout in Ca-free solution and the addition of a low Ca media (Fig. 2B). Fig. 2C again shows that placing the aortas in Ca-free solution for 10 minutes and then low Ca has no effect. However, if the aortas are placed in Ca-free solution with ouabain for 5 min-

utes, following which the tissue is washed in Ca-free solution without ouabain for an additional 5 minutes, an immediate contracture develops when the aortas are placed in a low Ca media (Fig. 2D). In Fig. 3, it can be seen that following a 15-minute wash in Ca-free solution, an immediate contracture develops when ouabain and Ca are added simultaneously, although Ca by itself (control) is not effective.

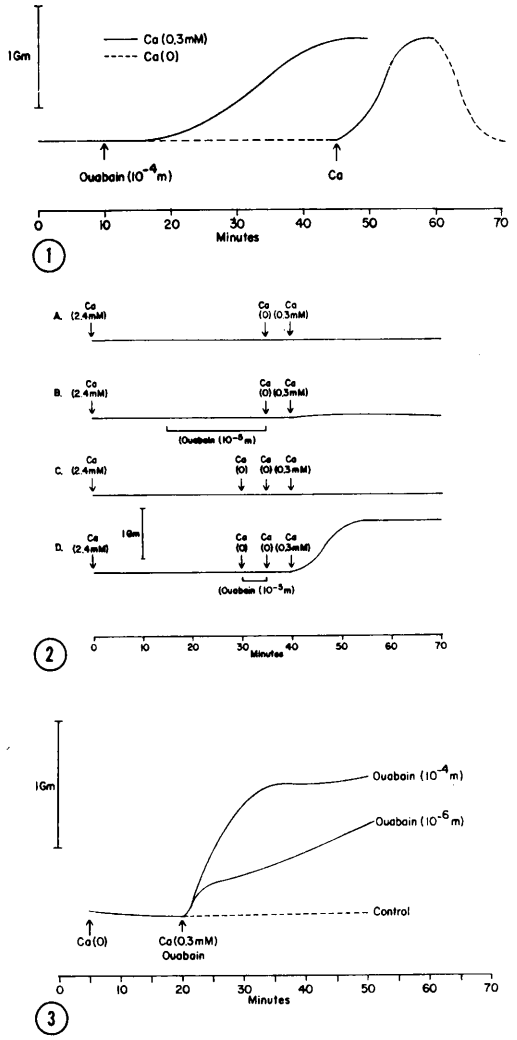

FIG. 1. Ouabain induced contracture in isolated rabbit aortic strips. Each line indicates a typical experiment obtained in 6-9 experiments.

FIG. 2. Effect of Ca on ouabain induced contracture in isolated rabbit aortic strips. Each line indicates a typical experiment obtained in 4-7 experiments. Arrows represent solution changes to the indicated Ca concentrations. The tissues were exposed to ouabain as indicated in brackets. The aortas were then exposed to ouabain and Ca-free solutions for 5 minutes before the low Ca solution was added (see *Results* for details).

FIG. 3. Ouabain induced contracture in isolated rabbit aortic strips. Solid lines indicate solution containing 0.3 mm Ca. Dashed lines indicate Ca-free solution. See *Results* for details. Each line indicates a typical experiment obtained in 6-7 experiments.

In Table I we present data on the effect of ouabain on Ca^{45} uptake, Ca content and Ca exchange. In preparations which have been preincubated for 2 hours in low Ca media followed by one additional hour in radioactive solution with or without ouabain, there is a significant ($p = .01$-$.001$) increase (38%) in Ca^{45} uptake, no significant change in Ca content and a significant ($p = .01$-$.02$) increase (50%) in Ca exchange in aortas with ouabain induced contractures as compared to controls without ouabain. Table I also shows Ca^{45} uptake in aortas preincubated in Ca normal solutions for 2 hours, Ca-free solutions for 15 minutes and then incubated in radioactive low Ca solutions for 30 minutes with or without ouabain 10^{-4} M and 10^{-6} M. Two sets of controls are presented since the experiments are paired (see *Methods*). Note that there is a significant increase in Ca^{45} uptake of 47% and 19% in aortas treated with ouabain 10^{-4} and 10^{-6} respectively. Ca contents were not determined under these conditions. The tension changes associated with these experiments are shown in Fig. 3.

Discussion. Considerable data have now accumulated which indicate that Ca is both intimately involved in contraction of vascular smooth muscle and in the action of the cardiac glycosides on the heart. Evidence is presented here which suggests that the action of the cardiac glycosides (ouabain) on vascular smooth muscle may also be mediated *via* a Ca mechanism in a manner which is similar to its action in heart muscle. Thus it can be shown that in Ca-free solutions ouabain does not produce a contraction (Fig. 1). Furthermore, there is a significant increase in Ca^{45} uptake associated with the contracture (Table I). Since the calcium content is not significantly altered, the increase in Ca^{45} uptake is considered to be due to an increased Ca exchange. Table I also indicates that

TABLE I. Effects of Ouabain on Ca^{45} Uptake and Ca Content in Isolated Rabbit Aortic Strips.

Conditions			Ca^{45} uptake	Ca content	Exchange
Preincubation	Incubation	Tension	(cpm/g × 10^4)	(meq/kg) wet tissue	(%)
Ca (.3 mM) (2 hr)	Ca (0.3 mM) (1 hr)				
	Control	0	(9) 7.34 ± .31 p = .01–.001	(8) 7.48 ± .70 p > .9	(9) 8.68 ± .39 p = .001
	Ouabain (10^{-4} M)	+	(9) 10.14 ± .79	(8) 7.52 ± .91	(9) 13.10 ± 1.18
Ca (2.4 mM) (2 hr)					
Ca (0) (15 min)	Ca (0.3 mM) (30 min)				
	Control	0	(6) 6.09 ± .44 p = .05–.02		
	Ouabain (10^{-4} M)	+	(6) 8.97 ± .69		
	Ca (0.3 mM) (30 min)				
	Control	0	(7) 7.54 ± .78 p = .05–.02		
	Ouabain (10^{-6} M)	+	(7) 9.00 ± .64		

The mean, ± SEM and p values are given. Number of experiments precedes the means in parentheses. Percentage exchange was calculated by using each individual Ca^{45} uptake experiment and the mean Ca content for each group.

under appropriate conditions, the increase in isotope uptake can be measured in aortas with concentrations of ouabain of 1×10^{-6} M.

The relationship between ouabain, Ca and tension development, however, is not a simple one, for Ca and ouabain appear to be antagonistic in certain aspects. Thus in Fig. 2 it can be seen that exposure of the aortic strip to ouabain or Ca-free solution separately has no effect on contraction (under the conditions indicated) but brief exposure to both does result in contraction when Ca is present. Furthermore, Fig. 3 shows that preincubating the aortas in Ca-free solutions for short periods increases the tissue sensitivity to the effects of ouabain so that concentrations of 1×10^{-6} will produce immediate rapid contractures when Ca is added. Concentrations as little as 5×10^{-7} can usually be detected in this manner. In contrast, when Ringer's solution containing the usual Ca concentrations (2.4 mM) is used, concentrations of ouabain of 10^{-4} or above must be employed in order to obtain rapid and consistent effects. Lower concentrations of ouabain (1×10^{-6}) can produce contractures in normal Ca media, but the effect is delayed and variable.

The antagonism between ouabain and Ca could be related to an effect on membrane polarization(12). We have recently been able to demonstrate that both ouabain (unpublished observations) and Ca-free solutions (submitted for publication) induce a gradual depolarization in the rabbit aortic strip. It is possible that an additive effect on depolarization may account for this increased sensitivity of the strip to ouabain after an initial brief exposure to Ca-free solutions. Furthermore, after depolarization by ouabain (and long exposures to Ca-free solutions) the tension developed by the aortic strip becomes very sensitive to changes in the extracellular Ca concentration (Fig. 1). This suggests that the depolarized aortic strip may become quite permeable to Ca and could account for the increased Ca^{45} exchange measured in this study.

These studies have indicated that vascular tissue is relatively resistant to ouabain *in vitro* and develops an increase in sensitivity to ouabain when placed in Ca-free solutions for short periods. It is of interest that the rat atrium which is also quite resistant to the action of the cardiac glycosides has a Ca exchange rate (7%)(13) similar to that of the rabbit aorta (8.68%—Table I) but approximately one-third (18%) of that of the ouabain sensitive guinea pig atria. Furthermore, the Ca concentrations of the ouabain

resistant rat atria(13) (11.61 meq/kg) and rabbit aorta (7.48 meq/kg, Table I) are higher than that of the sensitive guinea pig atria (5.2 meq/kg)(7). A precise comparison cannot be made since the experiments were not carried out under identical conditions. However, these data suggest the possibility that the mechanism(s) responsible for species variations in sensitivity to the cardiac glycosides may be Ca dependent and related to the level of intracellular Ca and/or the strength of Ca binding.

Summary. The contracture produced by ouabain in isolated rabbit aortic strips is Ca dependent and associated with an increase in Ca^{45} uptake, no change in Ca content and thus presumably, an increase in Ca exchange. Ca and ouabain are also antagonistic in that the sensitivity of the tissue to ouabain can be markedly increased by placing the aortas in Ca-free Ringer's solution for a brief period. It is suggested that species sensitivity to the cardiac glycosides may be related to the level of intracellular Ca and/or the strength of Ca binding.

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Emetic Action of Bacterial Endotoxin in the Cat.* (30758)

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Bacterial endotoxin provokes emesis in the dog(1), cat(2), monkey(3) and man(4) but information on the locus of emetic action is lacking. In the most pertinent study of this endotoxin action, cats vomited consistently following i.v. but not after intracerebroventricular injections of the lipopolysaccharide endotoxin (LPS). Ablation of the medullary chemoreceptor trigger zone, the site of emetic receptors for centrally acting emetics(5), did not influence the vomiting response to LPS.

The purpose of this communication is to compare the site of emetic action of LPS in the cat with that of the staphylococcus food poisoning agent, staphylococcal enterotoxin (6), as ascertained for this animal(7) and for the rhesus monkey(8). This was done by the functional approach(9), wherein the effect on the vomiting response to LPS of cats subjected to different denervation procedures was examined.

Materials. Escherichia coli 0111:B4 lipopolysaccharide endotoxin (Difco, control #457263) was used throughout. LPS stock solution was prepared in pyrogen-free saline by shaking after heating for 15 minutes in an 80°C water bath. Working dilutions were made from the refrigerated stock (kept up to 2 weeks) on day of use and heated for 7 minutes at 65°C.

About 70% of previously unused cats conditioned to the laboratory for 2-3 weeks and weighing 2-4 kg vomited in response to i.v. challenge of 5 μg/kg of LPS. Latency of the first vomiting episode was 25-70 min. Only those animals responding to this "standard-

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