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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izing" dose of LPS were used for denervation studies.

Highly purified (95% purity) staphylococcal Enterotoxin B elicited vomiting in about 50% of previously unused rhesus monkeys at a level of 10 μg/animal by the intragastric and with about 0.5 μg/kg by i.v. route. Six normal cats challenged i.v. with 1 μg/kg all vomited with latency of 45-120 minutes. To avoid the complication of degrees of resistance developing from prior contact with enterotoxin, cats not previously tested with enterotoxin were used directly for surgery and post-surgical challenges calculated on basis that they would normally have vomited after 1 μg/kg.

Intrathoracic vagotomy was performed below the level of the heart. Abdominal sympathectomy was accomplished in two stages: removal of the abdominal sympathetic chains followed by excision of the thoracic sympathetics below T4. When required, vagotomy was performed at time of the lower thoracic sympathectomy. Spinal cord transection was between T3 and T4.

All post-operative emetic challenges were performed only after apparent recovery from the surgical procedures (2-4 weeks) except that spinal-cord transected cats were tested 4-8 days after surgery. Food was offered immediately before administration of the emetic; animals not eating of own volition were given milk by gavage. Cats were observed for vomiting for 4-5 hours.

Results. Information on the resistance developing from previous LPS injections was developed in order to be able to quantitate the effect of denervation procedures on the emetic responsiveness of the cats. Daily injections of LPS induced a rapid increase in emetic tolerance. Two cats administered 5 μg/kg on 3 successive days vomited only after the initial challenge. Emesis was observed only after the first and third injections in a cat challenged with 5, 10, 20, 30, 40, and 50 μg/kg on succeeding days while another animal subjected to the same schedule followed by 70, 100 and 150 μg vomited after the first, third, and fourth injections only.

Significant emetic tolerance did not develop with spacing of LPS injections (Table I). Based on this observation, surgical procedures were scheduled so at least 3 weeks intervened between standardizing test and post-operative challenge. The latter dosage is reported as a multiple of the minimal emetic dose (MED) of 5 μg/kg, assuming no effect of the surgical procedure on the emetic responsiveness.

TABLE I. Failure to Develop Permanent Resistance to Emetic Action of LPS with Widely Spaced Challenges.

	LPS challenges*			
Cat No.	1st	2nd	3rd	4th
1	5+	10− (9)	5+ (30)	
2	10+	10− (9)	5+ (30)	5+ (21)
3	5+	10+ (21)	5+ (21)	
4	5+	5+ (21)	5+ (21)	
5	60+	10+ (21)		
6	60+	5− (12)	10+ (14)	5+ (21)

* μg/kg, i.v.; + = vomiting; − = no vomiting; ( ) days from preceding challenge.

Effects of denervation procedures on the emetic response to LPS are summarized in Table II. The results indicate that spinal cord transection interrupts the important afferent pathway of the vomiting reflex. However, abdominal deafferentation of vagotomy plus spinal cord sectioning is required to prevent all signs of an emetic response such as forward licking. This latter type of animal is still capable of vomiting in response to the proper emetic stimulus, vomiting resulting in 2 such cats given 180 μg/kg, i.v. of lanatoside C (Cedinilid, Sandoz), an emetic acting through the medullary chemoreceptor trigger zone(10).

Vagotomy of cats results in a low, but significant, increase in the threshold emetic dose of staphylococcal enterotoxin while complete resistance (maximum of about 10 MED's used) follows vagotomy plus high spinal cord sectioning(7). The data of Table II confirm these observations with higher enterotoxin doses and point out other similarities in emetic responses to these two toxins.

Discussion. As far as comparison is possible, the site of emetic action in the cat is the same for intravenously administered bacterial endotoxin and staphylococcal enterotoxin. The data are consistent with the inter-

TABLE II. Effect of Denervation Procedures on Emetic Response of Cats to LPS and Staphylococcal Enterotoxin.

Denervation	No. vomiting*/ No. tested	Dosage × MED†	Comments
		LPS	
Vagotomy	3/4	10, 2, 2, 1	1 MED cat not vomiting; latency 2-3 times normal
Vagotomy + abdominal sympathectomy	4/4	10, 10, 5, 3	Latency 2-4 times normal
Vagotomy + spinal cord (T3-T4)	0/6	5, 10, 20, 40, 50, 80	
Spinal cord (T3-T4)	0/5	4, 10, 20, 40, 60	Prodromal signs of vomiting with 20 and 60 MED's
		Staphylococcal enterotoxin	
Vagotomy + abdominal sympathectomy	2/3	5, 5, 5	
Vagotomy + spinal cord (T3-T4)	0/4	30, 60, 60, 80	
Spinal cord (T3-T4)	1/6	5, 10, 10, 20, 40, 50	Vomit with 50 MED's

* Total for all dosages.
† Estimated number of minimal emetic doses, assuming no influence of surgery.

pretation that these toxins induce emesis in the cat *via* action at the level of the abdomen.

Better evidence for the abdominal visceral origin of vomiting resulting from staphylococcal enterotoxin can be adduced for the monkey. Although destruction of the area postrema, the medullary structure considered to be the site of action of centrally acting emetics in the dog and cat, results in monkeys completely refractory to emetic action of staphylococcal enterotoxin(11), peripheral denervation gives equivalent tolerance. Intrathoracic vagotomy(12) and abdominal sympathectomy separately give incomplete protection but the combination of the two procedures produces monkeys no longer vomiting to either intravenous or intragastric challenges(8).

Requirement of high spinal cord transection rather than abdominal sympathectomy in conjunction with vagotomy for abdominal deafferentation necessary for complete protection of the cat against the emetic stimuli of the two toxins suggests a role for extrasympathetic afferents. Although the results differ from the present data in that cord sectioning without vagotomy had little effect on the vomiting response, the existence of such afferents has been indicated in the study of locus of emetic action of X-irradiation(13) and nitrogen mustard(14) in this animal species. These extrasympathetic afferents may possibly be the sensory innervation of the viscera with spino-bulbar tracts terminating in the vicinity of the medullary chemoreceptor trigger zone(15,16). This innervation would be interrupted by high spinal cord transection but not necessarily by abdominal sympathectomy. If this interpretation is correct, it must be assumed that in the experiments with staphylococcal enterotoxin these nerves were interrupted during abdominal sympathectomy of the monkey but not of the cat.

Tolerance to the emetic action of LPS develops rapidly. Although the "non-specific" nature of this tolerance was not investigated, the early appearance and the transitory duration of this refractory state resemble the sensitivity changes to the pyrogenic effects of bacterial endotoxin(17). A similar, rapidly developed, transitory tolerance to the emetic action of staphylococcal enterotoxin which is different from permanent immunity has been observed in monkeys fed enterotoxin(18).

Summary. Intravenously administered bacterial endotoxin (LPS) is an effective emetic for cats. The site of emetic action in this animal was studied by determining the emetic responsiveness to LPS following different denervation procedures. Intrathoracic vagotomy and vagotomy + abdominal sympathectomy had very little influence on subsequent response to LPS. High LPS challenge doses elicited only prodromal signs of vomiting in cats subjected to spinal cord transection at T3-T4. Complete tolerance to emetic action of LPS resulted from abdominal deafferentation produced by vagotomy + spinal cord sectioning. The changes in sensitivity of the cat to the emetic action of intravenously administered staphylococcal enterotoxin were comparable to that for LPS for the different types of denervation.

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Glucose, Lactate and Potassium Metabolism in the Isolated Perfused Rat Liver.* (30759)

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Advances in the technique of liver perfusion have provided investigators with a new and fascinating tools. The perfused liver can metabolize proteins, fats, and carbohydrates anabolically or catabolically with such facility that one is sometimes tempted to think of the *in vitro* organ as being an *in vivo* system (1-5). However, one limitation is that the perfused liver forms glycogen at a slower rate than it does in the intact animal(6). The present investigation was designed to find out if glycogen formation might be limited by electrolyte deficiencies or by an inability to utilize substrates other than glucose.

Methods. The perfusion apparatus and dissection methods were similar to those used by Miller *et al*(7). Modifications permitted simultaneous sampling of both the affluent and effluent perfusate, adjustment of perfusion pressure, and measurement of flow rate. pH electrodes were placed in the bottom reservoir, making it possible to know the hydrogen ion concentration throughout the course of perfusion. All tubing was siliconized to help

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