FIG. 5. Mature sporangium containing many large spores.
FIG. 6. Mature sporangium releasing endospores.
FIG. 7. a. Endospores germinating through sporangial wall. b. Immature sporangium produced by young hypha.
FIG. 8. Hyphal element serving as sporangium.

Further study on certain cytological aspects and on the exact nutritional requirements of the sporangial stage is in progress. Already it has been found that at least one strain will produce sporangia on the basic, chemically defined portion of the medium and on certain fractions thereof, though less satisfactorily than when coconut milk is added.

Summary. The *in vitro* cultivation of the parasitic phase of *C. immitis* is reported and the composition of the medium detailed. Sporangia were formed by all strains studied though in varying numbers and after varying periods of incubation. Mature culture-grown sporangia were indistinguishable from mature tissue sporangia.

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Locus of the Central Emetic Action of Cardiac Glycosides.* (18482)

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It has long been generally accepted that the so-called "central" emetic drugs act by direct excitation of the vomiting center. The concept was especially advanced by Hatcher and Weiss(1) who, in acute experiments, abolished the responsiveness of cats to a variety of emetic agents by making lesions in the dorsal vagal nuclei of the medulla. Also convincing was the demonstration by these workers(1,2) that the direct application of certain "central" emetics to the region of the ala cinerea induced vomiting in dogs and cats.

The premise that drugs act directly on the vomiting center is highly contingent on the certainty of the location of this center. That the dorsal vagal nuclei do not constitute the vomiting center was first indicated by the work of Koppanyi(3). He showed that dogs with chronic lesions in the ala cinerea continued to vomit in response to orally administered irritant emetics in spite of a concomitant refractoriness to the "central" emetic, apomorphine. On the basis of prevailing thought, Koppanyi interpreted his findings as due to incomplete exclusion of the vomiting center. The location of the vomiting center and its physiological role have been adequately delineated only recently by Borison and Wang(4) and Wang and Borison(5). They have demonstrated that the vomiting center, localized in the lateral reticular formation of the medulla, is quite distinct both anatomically and physiologically from the superficial region of the ala cinerea which was shown by Hatcher and Weiss to be sensitive to direct application of certain emetics and which was erroneously designated by them

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1. Hatcher, R. A., and Weiss, S., *J. Pharm. and Exp. Therap.*, 1923, v22, 139.

2. Hatcher, R. A., and Weiss, S., *Arch. Int. Med.*, 1922, v29, 690.

3. Koppanyi, T., *J. Lab. and Clin. Med.*, 1930, v16, 225.

4. Borison, H. L., and Wang, S. C., *J. Neurophysiol.*, 1949, v12, 305.

5. Wang, S. C., and Borison, H. L., *Arch. Neurol. and Psychiat.*, 1950, v63, 928.

as the emetic center. The question that remains is how this area in the dorsal surface of the medulla is associated with the nervous regulation of vomiting. The present report is concerned firstly with the characterization of a chemoreceptor trigger zone for emesis which is situated in the dorsal region of the ala cinerea and which serves as an afferent station for the vomiting center. Secondly, it is our purpose to show that the primary site of emetic action of intravenously administered digitalis glycosides is a specialized area in the central nervous system, namely, the chemoreceptor trigger zone for emesis.

Methods. In dogs anesthetized with pentobarbital and maintained with artificial respiration, a small bilateral lesion was placed by means of electrocautery in the caudal margin of the floor of the IVth ventricle. The emetic responsiveness of these animals was tested with apomorphine, copper sulfate and the cardiac glycosides lanatoside C, scillaren A and ouabain.† Postoperative responses were compared with the results of preoperative tests on the same dogs or with responses elicited in normal dogs given equivalent doses of the drugs. The tests were performed repeatedly during an observation period which extended for as long as 6 months postoperatively. The brains were perfused *in situ* with formalin when the animals were sacrificed, and the lesions were examined histologically.

Results. Apomorphine. Apomorphine hydrochloride was used to determine the success of the operation. A normal dog usually vomits after 0.01 mg per kg of apomorphine given intravenously and almost invariably responds to 0.02 mg per kg. Emesis occurs in about 2 minutes. In contrast, a dog in which a successful operation has been performed fails to vomit even to 1.0 mg per kg apomorphine. In approximately 1000 tests performed on more than 100 normal unanesthetized dogs, there was not a single instance when more than 0.03 mg per kg apomorphine was required to elicit vomiting. The drug was rapidly injected intravenously in 1.0 ml of a freshly prepared aqueous solution. A total of 11 dogs was subjected to the operation for destruction of the chemoreceptor trigger zone. Nine of these dogs proved to be completely and permanently refractory to apomorphine up to the limits of the doses tested, that is, 0.1 to 1.0 mg per kg.

Copper sulfate. For comparison with the "centrally" acting emetic drugs, copper sulfate was used as a gastrointestinal irritant for the purpose of eliciting vomiting through known reflex pathways(6). The oral administration of approximately 4.0 mg per kg (calculated as the anhydrous salt) copper sulfate was found to be effective for inducing vomiting in most normal dogs. In no case, in more than 100 dogs tested, was it necessary to use over 8.0 mg per kg. The copper sulfate was dissolved in 50 ml of distilled water and administered by stomach tube after one day of food deprivation. The average latent period for vomiting was 19 minutes. The 9 dogs made refractory to apomorphine by operations on the medulla did not show any significant change in responsiveness to copper sulfate. In fact, these dogs had no detectable deficit in the functional character of the vomiting in response to copper sulfate. Except for refractoriness to intravenous apomorphine, the chronic operated dogs showed no overt signs of central nervous system damage and could not be distinguished from normal dogs. Thus it has been established that the central mechanism for vomiting in response to irritation of the enteric tract by copper sulfate is intact in chronic operated dogs which are unable to respond to the very potent emetic drug, apomorphine.

Cardiac glycosides. Three cardiac glycosides, namely, lanatoside C, scillaren A and ouabain, were tested for emetic action in the dogs with lesions in the chemoreceptor trigger zone. Preliminary tests were made on normal dogs with each of the drugs to determine the emetic dose effective in all animals.

† Lanatoside C and scillaren A were kindly supplied by Mr. Harry Althouse and Mr. S. M. Fossel of Sandoz Chemical Works, Inc., and ouabain by Dr. K. K. Chen of Eli Lilly and Co.

6. Wang, S. C., and Borison, H. L., *Am. J. Physiol.*, 1951, v164, 520.

TABLE I. Emetic Activity of Certain Cardiac Glycosides Injected Intravenously.

Drug	Dose, mg/kg	Normal dogs		Operated dogs*	
		Tested	Vomited	Tested	Vomited
Lanatoside C	.05	9	3	—	—
	.07	5	2	—	—
	.08	13	13	3	0
	.10	12	12	6	0
Scillaren A	.05	6	0	—	—
	.07	8	3	—	—
	.08	5	5	—	—
	.10	5†	5	5	1‡
Ouabain	.03	3	1	—	—
	.04	3	1	—	—
	.06	3	3	2	0

* Refractory to apomorphine inj. intravenously.
† One dog died.
‡ Vomited 6 hr after inj.

The glycosides were administered by the intravenous route in a small volume of 5 to 50 per cent alcohol, each ml containing from 0.15 to 1.0 mg of the drug. When vomiting was elicited in the normal animal, the first expulsion of vomitus generally occurred within 15 minutes following injection of the drug. The data are summarized in Table I. Only one of the 9 successfully operated dogs vomited in response to doses of the cardiac glycosides shown to be effective in all normal dogs tested. This single instance of vomiting in an operated animal was in response to scillaren A and the latent period was 6 hours. Effects of the drugs at dose levels higher than those indicated in Table I were not studied in this series of experiments.

Anatomical localization. The most accurate description of the site of the chemoreceptor trigger zone for emesis which can be given at present is based solely on topographical relationships of the lesion to known medullary nuclei. In a composite appraisal of all specimens, the area of ablation was observed histologically to be a very small superficial pocket situated in the dorsal region of the ala cinerea. Only minimal damage was found in the vagal nuclei. To the authors' knowledge, the region herein localized has not previously been analyzed morphologically. This study is now in progress and the findings will be published in a separate paper.

Discussion. Investigations on the mechanism of digitalis-induced vomiting have resulted in a highly controversial literature. For a good survey of the subject, the reader is referred to Dresbach(7). This worker very painstakingly interrupted as far as possible every known visceral afferent pathway to the medulla oblongata without eliminating digitalis emesis. Although all recent indications have pointed to a central emetic action of digitalis, the exact site of this action has never been demonstrated. The isolated observation made by Koppanyi(3) that intravenous digitalis failed to induce emesis in two dogs with chronic lesions in the ala cinerea went unheeded despite its potential significance. Both of his animals had rather widespread but incomplete destruction of the dorsal vagal nuclei. His results were undoubtedly the consequence of diffuse damage which included the chemoreceptor trigger zone located in the dorsal region of the ala cinerea.

On the basis of our earlier work on the vomiting center(4,5) and the present experiments on the central site of action of digitalis, a new concept of the medullary regulation of vomiting has been evolved. According to this concept, drugs such as apomorphine and digitalis glycosides do not exert a direct action on the emetic center. Vomiting is initiated through reflex circuits, regardless of whether the receptor site is peripheral, as in the gastrointestinal mucosa, or central, as in the chemoreceptor trigger zone of the medulla. It is interesting that Hatcher and Weiss (cited by Hatcher)(8), after observing the sudden inhibition of vomiting by the scratch and defecation reflexes, concluded that vomiting is purely reflex in nature.

The present delimitation of the central locus of emetic action of the cardiac glycosides by no means excludes the possibility that reflex vomiting may be initiated at certain peripheral sites by these drugs. Indeed, the fact that a delayed emetic response to intravenous scillaren A was observed in one

7. Dresbach, M., *J. Pharm. and Exp. Therap.*, 1947, v91, 307.
8. Hatcher, R. A., *Physiol. Rev.*, 1924, v4, 479.

successfully operated dog suggests that there may be a site of emetic action for the cardiac glycosides other than in the medulla.

As far as we are aware, the trigger zone for emesis in the medulla oblongata represents the first such discrete and specialized chemoreceptor area clearly demonstrated to reside within the central nervous system. Speculation on this discovery suggests certain broad implications for chemoreceptor physiology in general, and the need for re-evaluation of "centrally" acting drugs in particular.

Summary. Experiments on dogs have revealed the existence of a chemoreceptor trigger zone for emesis which is quite distinct from the vomiting center. This zone is a bilateral structure situated at the surface of the medulla oblongata in the dorsal region of the ala cinerea. Destruction of the emetic chemoreceptor zone results in animals which are permanently refractory to apomorphine given by vein, but it does not impair the vomiting response to orally administered copper sulfate. Eight out of 9 such dogs failed to vomit when tested intravenously with known emetic doses of the cardiac glycosides, lanatoside C, scillaren A and ouabain. Only one vomiting response was elicited; in this case, the latent period was greatly prolonged, a fact which suggests the existence of another site of emetic action of digitalis glycosides. The significance of a central chemoreceptor trigger zone for the emetic action of digitalis is discussed.

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Effect of Renal Decapsulation on Hypertension Induced by Single Episode of Acute Choline Deficiency.* (18483)

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When weanling rats are fed a properly devised choline deficient ration, within one week they develop renal lesions characterized grossly by subcapsular bleeding so that the syndrome has been called simply "hemorrhagic kidneys"(1). If at this time the rats are transferred to stock rations, many of them recover. However, over the course of several months these animals develop systemic arterial hypertension of varying degree(2,3). Since the earliest descriptions of the "hemorrhagic kidney syndrome" noted that the kidneys of those animals which survived the acute episode appeared "frosted"(1), presumably denoting a fibrotic capsule, it seemed possible that the pathogenesis of hypertension in these rats might be similar to that in animals in which hypertension has been induced by coating the kidney with silk, cellophane, acrylic resin or other plastic material. This would seem particularly likely if such a fibrotic capsule is unable to grow at a rate commensurate with that of the renal parenchyma. The present study was designed to test this hypothesis.

Experimental. The experimental animals were males of the Vanderbilt strain(4). When they had attained a weight of 40 g they were housed in individual wirebottomed cages and fed the choline deficient diet(3) *ad libitum*. After 6 days, each rat was given a single dose of 10 mg of choline chloride by pipette and returned to a commercial stock

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1. Griffith, W. H., *Biol. Symposia,* 1941, v5, 193.

2. Best, C. H., and Hartroft, W. S., *Fed. Proc.,* 1949, v8, 610.

3. Handler, P., and Bernheim, F., *Am. J. Physiol.,* 1950, v162, 189.

4. Wolfe, J. M., Bryan, W. R., and Wright, A. W., *Am. J. Physiol.,* 1938, v34, 352.