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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ration.† Of 58 rats so treated in the present study, 9 died of the acute renal lesions. One week later, both renal capsules were stripped and removed from 13 of the surviving rats (Group A) under sodium pentobarbital anesthesia and the kidneys replaced in their normal position. Care was taken not to rupture the peritoneal wall.

A second group of 12 choline deficient animals (B) was allowed to develop hypertension and, 12 weeks after the acute renal episode, their kidneys were decapsulated in similar fashion. A third group of choline deficient animals (C) served as controls. As further controls another group of 12 rats (D) were fed stock ration throughout, the choline deficient period being omitted, but renal decapsulation was performed when they weighed 125 g. Four weeks after the initial choline deficient period, all rats attained a size sufficient to permit satisfactory estimations of systolic blood pressure with a modified version of the end-point device of Skeggs and Leonards (5) similar to that of Chittum, Hill and Grimson(6) Weekly blood pressure estimations were made, thereafter, and the results are shown in Fig. 1. The values plotted represent the simple arithmetic mean of all animals in each group.

As found previously, virtually all rats subjected to the episode of choline deficiency became hypertensive in varying degree although few rats showed pressures exceeding 160 mm after 16 weeks. This may have been due merely to the relatively brief duration of these experiments. Decapsulation immediately following choline deficiency (Group A), resulted in an actual elevation in pressure during the first few weeks of the experiment. However, as seen in Fig. 1, this transient hypertension was independent of the choline deficiency experience since it was also observed in Group D. The effect was relatively short lived and after 10 weeks the blood pressures of both groups subsided to normal

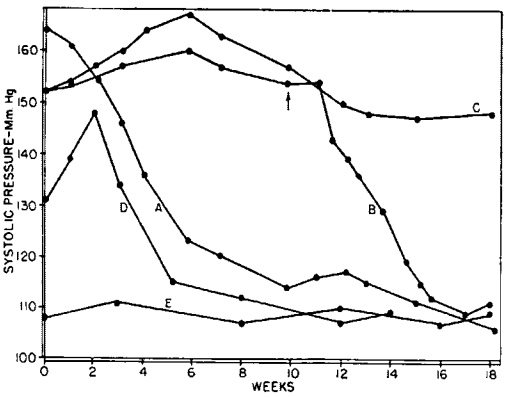

FIG. 1.

Effect of renal decapsulation on hypertension due to hemorrhagic kidney syndrome. Groups A, B, and C were choline deficient for a one week period, 3 weeks before 0 time, then were maintained on chow. The kidneys of group A were decapsulated one week after the choline deficiency episode, while those of group B were decapsulated at time indicated by the arrow. Group C was not decapsulated. Group D was not choline deficient, but was decapsulated one week before 0 time. Group E were normal controls.

levels and remained there for the duration of the experiment. As seen from the behavior of group B, renal decapsulation not only prevented the hypertension which is ordinarily observed subsequent to choline deficiency, but also abolished such hypertension after it had been established.

It had previously been shown that the hypertension of partially nephrectomized rats disappears when they are fed a low protein diet(7) and that the hypertension can be restored by the administration of a dose of ACTH which is without effect on the blood pressure of normal rats(8). It seemed of interest, therefore, to determine the effect of ACTH on the blood pressure of the decapsulated rats described above. In Fig. 2 is shown the effect of single, daily, intraperitoneal doses of 1 mg of ACTH‡ to rats of groups A, C, D, and E during the 14th week

† Ralston-Purina Co., St. Louis, Mo.

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‡ Obtained from Armour and Co., Chicago, Ill., through the courtesy of Dr. J. D. Fisher. This preparation was from lot 60-61 and was stated to have a biological potency of 100% of standard La-I-A.

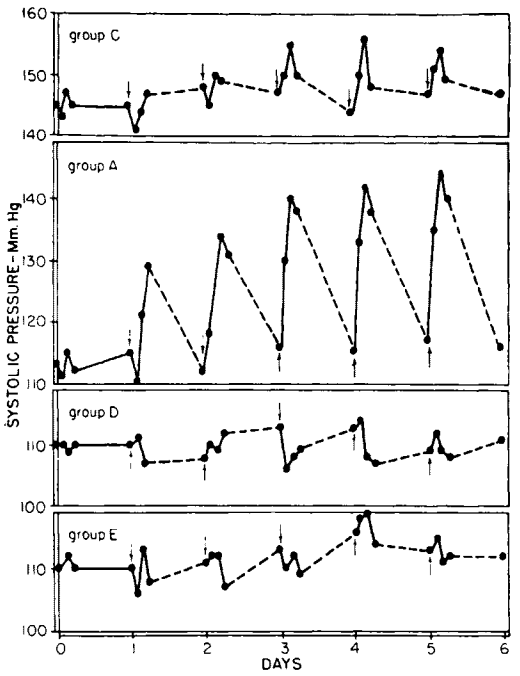

FIG. 2.

Effect of ACTH on blood pressure of renal decapsulated rats. For explanation of groups see text and Fig. 1. ACTH (1 mg) was given at times indicated by the arrows.

of the experiment. While this treatment did not affect the normal rats of groups D and E or the hypertensive rats of group C, it effectively induced hypertension in the erstwhile choline deficient group in which hypertension had been prevented by renal decapsulation.

At the termination of these experiments, the kidneys of 9 of the animals in group A and 6 each from groups C, D, and E were fixed in formaldehyde. These were examined histologically by Dr. R. H. Follis, Jr.§ of the Johns Hopkins School of Medicine who reported, "There are no changes in the parenchyma or vessels of any group which set them apart from the others. Group C does show thicker capsules than the other capsulated kidneys (groups D and E) while no capsules were apparent in group A. In no case did there appear to be evidence of an excess vascular supply."

Discussion. It is apparent from these data that renal decapsulation can both prevent and alleviate the hypertension ordinarily consequent upon a single episode of acute choline deficiency. No simple explanation is at hand to account for this relationship between the renal capsule and blood pressure. However, these findings are compatible with the hypothesis that the hypertension under consideration may be initiated by an inability of the renal capsule to grow normally after recovery from the "hemorrhagic kidney syndrome". After this work was completed there appeared the report of Danford, *et al.*,(16) which demonstrated that surgical removal of the silk capsule from the kidneys of rats in whom hypertension had been induced by this means resulted in a fall in blood pressure to normal values in 3 of 4 animals. Thus, the analogy appears quite complete. Hartroft(17) has found that renal decapsulation, performed *prior* to the development of 'hemorrhagic kidneys' does not prevent or alleviate this syndrome. As the rats did not survive the acute disease, this author could not observe the subsequent effect of decapsulation on blood pressure. In the present work, however, renal decapsulation was performed 1 and 12 weeks *after* the acute episode of 'hemorrhagic kidneys', yet was effective in interrupting the events ordinarily consequent upon this acute disease.

Renal decapsulation has also been found to alleviate the hypertension of dogs with artificially constricted renal arteries(9,10) although this procedure has not proved beneficial in human hypertensives(11-13). However, the concept involved in these latter studies was of a different nature. Previous

§ We are especially indebted to Dr. Follis for his kindness and cooperation.

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workers have been careful to cover the decapsulated kidney with omentum so as to develop a collateral renal circulation(14). In the present experiments, after decapsulation, the kidneys remained in their normal anatomical relationships. At autopsy the decapsulated kidneys were surrounded with the usual perirenal adipose tissue and there was no evidence of any collateral circulation. However, the decapsulated kidneys did present rather bizarre, lobulated forms and were invariably somewhat larger than the normal kidneys of corresponding animals, both choline deficient and non-choline deficient.

Whatever may be the role of the capsule in this situation, it cannot be the sole factor involved. As shown by the effects of ACTH, even after decapsulation, there remains considerable functional impairment. It has been found in this laboratory that ACTH, in this same dosage, will restore the hypertension of rats from which ¾ of their renal tissue has been removed and in whom hypertension has been prevented by feeding a low protein diet(8) and will also produce hypertension in bilaterally nephrectomized rats while they survive(15), yet is without effect on unilaterally nephrectomized rats. Thus, the renal decapsulated, normotensive post-choline deficient rats of the present study may be considered to have, in this sense, renal function comparable to that of an animal with somewhat less than one intact kidney. There may be an analogy between such rats and humans who develop hypertension under stress or 'tension' yet appear to have normal renal function by the usual criteria.

Summary. Bilateral renal decapsulation, performed one week after an acute episode of 'hemorrhagic kidneys' in choline deficient rats, prevents the development of hypertension in such animals and restores blood pressure to normal when the operation is performed in rats in which such hypertension is already established. Administration of ACTH to such renal decapsulated rats restores their hypertension but is essentially without effect on the pressures of normal rats, hypertensive rats, or renal decapsulated rats which never were choline deficient.

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A Microbiological Assay Method for Biotin. (18484)

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Microbiological methods for the determination of biotin have been employed in many laboratories using several microorganisms (*Lactobacillus casei, L. arabinosus, Clostridium butylicum, Leuconostoc mesenteroides, Rhizobium trifolii*)(1,2). Although 2 molds (*Neurospora crassa* wild and cholineless, and *Nematospora (Ashbyia) gossypii*) have been used as test organisms for biotin(3-5) they have not yet been generally used.

In the present paper a new method is described based on the biotin requirement of the filamentous fungus *Allescheria boydii*. This observation was first reported by Cury (6) and a more detailed study on vitamin nutrition related to biotin was published by Villela and Cury(7). It has been shown that *A. boydii* is a specific organism for biotin and that the growth responses are proportional to the amount of this vitamin in the medium.

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