striking decrease was found with the *L. casei* and *P. cerevisiae* assays. The results indicate an almost complete depletion of F.A. content of the red cells.

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Cardiac Tissue Response to Endotoxin.* (27333)

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Myocardial injury is considered to play a primary role in the development of irreversibility to transfusion for hemorrhagic shock (1). The evidence for such injury is biochemical and functional data that allow various interpretations. There is no morphologic evidence for such injury, in spite of the fact that the catechol amines and bacterial toxins, both of which are present in excessive amounts in the blood of the animal in hemorrhagic shock, produce morphologic injury in the myocardium and other tissues of normal animals(2,7). The absence of such morphologic evidence in the shocked animal might be because it cannot react to injury. To test this hypothesis experiments were performed to see if the morphologic pathology caused by these substances in the normal animal can also develop in the shocked animal that is allowed to recover and survive long enough to allow the reaction to injury to become manifest.

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Materials and methods. Hybrid albino adult rabbits were used. Sublethal doses of *E. coli* endotoxin (0.5 mg/kg) or 0.1 mg/kg of norepinephrine or epinephrine were given intravenously singly or together. The experiments were repeated in rabbits that had received dibenamine (25 mg/kg) intravenously 3 hours in advance. Eleven rabbits were bled to produce reversible hemorrhagic shock of 2 hours duration, and were given 100 μg of endotoxin just before transfusion. These rabbits did not receive catechol amines because the blood concentration of these substances is already more than adequate in shock(7,8).

All rabbits which were in apparently good condition 48-72 hours later were killed. In addition to routine postmortem examination, tissue sections of lung, liver, spleen, kidney, and heart, fixed in 10% neutral formalin, were prepared and stained with hematoxylin and eosin or with the von Kossa stain, and examined microscopically.

Results. (Table I). A. Normal rabbits. Six animals which received neither endotoxin

TABLE I. Effect of Endotoxin, with and without Catechol Amines, upon Myocardium in Normal, Dibenamine-Pretreated, and Reversibly Shocked Rabbits.

				Endotoxin and		
	Control	Endotoxin	Norepinephrine	Norepinephrine	Epinephrine	Reversible shock
No. of rabbits	6	18 (9)	5 (4)	12 (7)	10 (7)	11
Cardiac damage—Severe*	0	2 (0)	0 (0)	6 (1)	7 (0)	6‡
Mild†	0	7 (0)	3 (0)	4 (3)	3 (3)	2

All figures in parentheses = experiments in dibenamized rabbits.

* Severe—more than 25% of one ventricle involved.

† Mild— less " " " " " " .

‡ All rabbits were killed after 48 hr except 3 in the shocked group. These died in less than 8 hr and their hearts showed only severe congestion.

nor catechol amines showed only normal histology (Fig. 1A).

The myocardial tissue response to catechol amines given alone was mild or absent. The response to endotoxin given alone varied with the dose. In most instances the damage was somewhat more extensive and severe than that caused by the catechol amines. When endotoxin was administered with epinephrine or norepinephrine the incidence of damage was much higher (over 80%), and the reaction was more severe, especially in those receiving epinephrine.

The cardiac damage produced by the combined action of endotoxin and catechol amines may be summarized as follows: Gross cardiac abnormality was unusual. In a few instances the heart was dilated, and on cross section showed a thinner than normal ventricular wall. Microscopy revealed an inflammatory and necrotizing reaction in the myocardium (Fig. 1B & 1C). Occasionally the inflammatory reaction involved the entire myocardium, but in most instances it was focal. As a rule both ventricles were involved; but the injury was more frequent and more extensive in the right ventricle and interventricular septum than in the left ventricle. In the latter the papillary muscles were frequently affected. The endocardium and epicardium were intact, and valvular lesions were not observed.

The reactive cells were almost exclusively mononuclear—chiefly histiocytes. Plasma cells, neutrophils and lymphocytes were rare. The interstitium was edematous. The myocardial fibers were separated and shrunken, and occasionally totally replaced by the histiocytes. There was a varying degree of connective tissue proliferation. Tissue necrosis was always focal, and calcification was proportional to the extent and severity of the necrosis (Fig. 1C). Vascular thrombosis or necrosis was not observed. The coexistence of inflammatory reaction and necrosis in variable proportions suggests that they are related processes.

The microscopic changes in the organs other than the heart were slight to absent, and did not bear any relation to degree of myocardial injury, or to the type of experiment. The spleen was normal. Small hemorrhagic foci in the lungs were found in about a quarter of the animals. Rare fibrin thrombi were present in the small pulmonary arteries of 3 dibenamine-treated rabbits. Focal coagulative necrosis was found in some 10% of the livers examined. Two rabbits showed extensive cortical necrosis of the kidney. Of these one had received one sublethal dose of endotoxin; the other had the same dose of endotoxin plus norepinephrine.

Dibenamine prevented the injury caused by endotoxin given alone, or catechol amines given alone. It also prevented the severe reaction produced when these are given together. Mild reactions, however, were not prevented.

B. Rabbits in reversible shock. Of the eleven rabbits receiving endotoxin just before the transfusion for 2 hours of shock, three died in less than 24 hours, and eight survived 48 hours. As expected, the three which died in less than 24 hours showed no lesion. The eight which survived for 48 hours did show the lesion produced in normal rabbits by the

combined use of endotoxin and catechol amines.

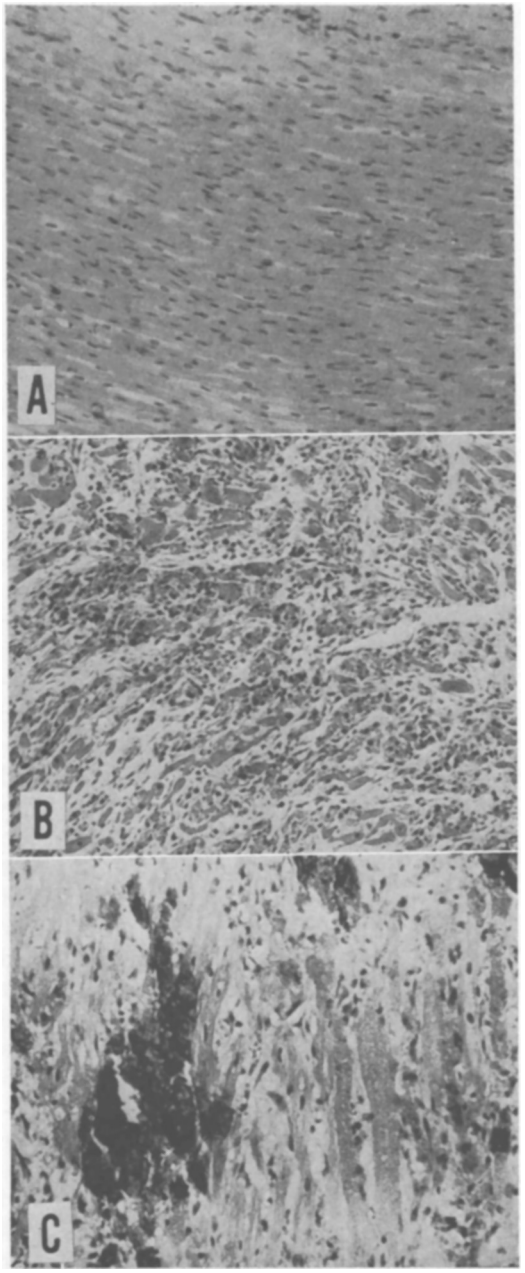

FIG. 1. A. Normal myocardium. B. Combined action of endotoxin and catechol amines. An area of myocarditis showing infiltration by mononuclear cells, edema of interstitial tissue and fragmentation of myocardial fibers. Hematoxylin and eosin stain. C. Combined action of endotoxin and catechol amines. An area of necrosis showing swollen necrotic myocardial fibers, focal calcification and slight mononuclear response. Hematoxylin and eosin stain.

Discussion. Because all six control animals showed normal gross and microscopic anatomy, the changes observed on giving endotoxin or catechol amines may be properly attributed to these substances. The increase in the extent and severity of the myocardial damage produced by the combination of endotoxin with epinephrine or norepinephrine, and the protective effect of dibenamine, support the observation of Thomas(8) that endotoxin potentiates the action of the catechol amines, and that the morphologic injury is caused by the amines rather than the endotoxin. Hinshaw *et al.*(9) could find no support for this hypothesis in a study of the functional effect of endotoxin on the response of pulmonary or forelimb blood vessels to epinephrine. But the hypothesis does not require that a significant functional injury be present in all tissues. The phenomenon, as first described by Thomas, relates to a morphological injury of skin. Our data confirm the hypothesis with respect to the myocardium.

Although catechol amines were not needed to produce the lesion in the shocked animal, one may assume that endotoxin cannot act independently, for they are present in ample quantity in the blood and myocardium of the shocked animal(10,11). The observation that the generalized Shwartzman reaction requires an intact sympathetic nerve supply also shows that endotoxin acts *via* the adrenergic system(12).

Summary and conclusion. Administration of catechol amines together with endotoxin to normal rabbits results in a greater degree of inflammation and necrosis of cardiac muscle than is produced by either agent alone. The damage is prevented or markedly reduced by pretreatment with dibenamine. It can be produced by endotoxin in shocked animals without the catechol amines, presumably because the amines are already present in high concentration. Although the data obtained do not demonstrate that a specific cardiac lesion attributable to endotoxin is a key factor in development of irreversibility to transfusion in hemorrhagic shock, they are not inconsistent with such a hypothesis.

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Recovery of Thiamine Injected into the Rat Cecum.* (27334)

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Thiamine is synthesized by the microbial flora of the simple stomach animal and evidence has been presented that this biosynthesized thiamine cannot be absorbed from the large intestine of the rat(1,2,3). It is possible that lack of absorbability is due to the form of the newly synthesized thiamine; for example, it has been noted that thiamine is held within the bacterial cell(4). Recently Wostmann and Knight(3) have shown that thiamine synthesized in the large intestine is present largely in a form that remains in the centrifugal supernatant of the cecal contents and, therefore, is probably extracellular. This thiamine was not utilized by the host animal. It was of interest then to see if thiamine in water solution, injected directly into the rat cecum, could be absorbed.

Materials and methods. Two separate experiments were conducted. In the first, 3 groups of 6 male weanling rats (Holtzman) were fed a purified diet devoid of thiamine for 2 weeks. One animal died in Groups 1 and 2, leaving 5 rats in these groups. Coprophagy was prevented in all rats by plastic tail cups(5) and rats were housed individually in raised wire bottom cages. In Group 3 (Table I) 50 mg of procaine penicillin was added per kilo of diet. On the first experimental day all rats were anesthetized with ether and the cecum exposed through a midline abdominal incision. Using a tuberculin syringe with a No. 26 needle introduced through the wall of the cecum, Group 1 received 0.2 ml of water and Groups 2 and 3 received 140 μg thiamine hydrochloride in 0.2 ml water. On the eighth day the operative procedure was repeated, but this time 0.1 ml was injected into the cecum; Group 1 receiving water and groups 2 and 3, 82 μg thiamine hydrochloride. Feces were collected daily and total thiamine determined by the thiochrome method as previously described (1). A control series was run with 3 groups of 10 rats each. The rats were fed the purified diet devoid of thiamine for 2 weeks. Coprophagy was prevented in all rats. On the first experimental day all rats were anesthetized with ether and the cecum exposed through a mid-line abdominal incision. This was a sham operation for only 0.2 ml of water was injected into the cecum. After the incision had been closed the 3 groups were given, by stomach tube, 1 ml of water, 7 μg thiamine hydrochloride, 14 μg thiamine hydrochloride. This procedure, including the sham operation, was repeated on the eighth experimental day.

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