5. Charlton, R. W., Jacobs, P., Seftel, H., Bothwell, T. H., Brit. Med. J., 1964, Dec. 5, 1427.
6. Forrester, R., Conrad, M., Jr., Crosby, W., Proc. Soc. Exp. Biol. and Med., 1964, v111, 115.
7. Wilson, T. H., Wiseman, G., J. Physiol., 1954, v123, 116.
8. Brown, E. G., Jr., Justus, B. W., Am. J. Physiol., 1958, v194, 319.

Received July 13, 1965. P.S.E.B.M., 1965, v120.

Streptococcal and Staphylococcal Endocarditis and Renal Lesions in Rats After Epinephrine in Oil and Dibenamine. (30664)

BENJAMIN HIGHMAN AND PAUL D. ALTLAND
Laboratories of Experimental Pathology and of Physical Biology, National Institute of Arthritis and Metabolic Diseases, NIH, PHS, U. S. Dept. of H.E.W., Bethesda, Md.

Epinephrine in oil, 5 mg/kg subcutaneously daily in rats, produced a myocarditis, usually most pronounced 24 hours after the second dose(1). The myocarditis was associated at times with a severe valvulitis and with sterile vegetations involving the left atrium and occasionally the left ventricle. These changes were prevented or mitigated by prior treatment with an adrenergic blocking agent such as Dibenamine hydrochloride. The purpose of this study was to determine the susceptibility of such rats to bacterial endocarditis and the effect of pretreatment with Dibenamine.

Materials and methods. Five lots of young male Sprague-Dawley rats, 90-100 days of age and 250-350 g in weight, were housed, 4-6 per metal cage, and given a Purina Laboratory Chow diet and water *ad libitum*. Each lot included about 50 rats and was divided into 3 groups. All rats in the 3 groups were given in random order on the same day or on 2 successive days, an injection in the tail vein of 0.5 ml of a 5-hr specified bacterial broth culture(2). One control group received only bacteria. Another group received 2 subcutaneous doses of 5 mg/kg epinephrine in oil at 24-hour intervals, one dose 24 hours before and the second immediately after the first bacterial injection. The third group was treated like the second except that the rats were given in addition, 2 subcutaneous doses of 50 mg/kg of Dibenamine hydrochloride (N-(2-Chloroethyl) dibenzylamine Hydrochloride), one dose 48 and one 24 hours before the first dose of epinephrine in oil.

The bacteria selected were *Streptococcus mitis*, JH-26 and *Staphylococcus aureus*. In previous studies, it was found that intravenous injection of cultures of these bacteria produced a low incidence of endocarditis in normal animals and a high incidence in animals rendered susceptible by prior exposure to simulated high altitude(2,3,4,5) or a cold environment(6) or by prior surgical induction of aortic insufficiency(7). In the initial experiments, the animals received one injection of *S. mitis*. Since the organisms appeared less virulent after repeated subcultures, the rats in later experiments were given a broth culture of *S. mitis* or *Staph. aureus* on each of 2 successive days.

Autopsies were made on surviving rats 6 days after the first bacterial injection and on those that died after surviving at least 48 hours, a period sufficient for development of bacterial endocarditis(5). The heart and kidney were fixed in a 10% aqueous solution of buffered (pH 7.0) formalin. After fixation, the heart was bisected frontally through all 4 chambers. Paraffin sections were prepared in a routine manner. Skip serial sections from each half of the heart and sections of the kidney, made through the hilus and grossly visible lesions, were stained with azure eosin.

Results. The results are summarized in Table I. The mortality rate in rats given epinephrine in oil and *S. mitis* was significantly greater statistically, if the 2 streptococcal groups are combined, than in control rats given *S. mitis* alone; the mortality rate was significantly greater also in rats given epi-

TABLE I. Incidence of Lesions in Rats after I.V. Inoculation of Bacterial Cultures.

Group*	No. of rats	Mortality (%)	Incidence of endocarditis: Bact lesions No.	Bact lesions %	Total No.	Total %	No. of rats with lesions: Site of bacterial vegetations: Mitral	Aortic	Tricuspid	Mural	Interstitial nephritis	Bact papillitis
After single inoculation of *Streptococcus mitis*, JH-26 culture												
C	30	0	1	3‡	5	17‡	0	1	0	0	20	2
E	36	15	8	22	19	53	6	1	2	4	22	6
DE	43	4	1	2‡	6	14‡	1	0	0	0	29	5
After 2 inoculations of *Streptococcus mitis*, JH-26 culture												
C	29	0†	1	3†	5	17†	1	0	0	0	20	1
E	29	20	8	28	19	66	6	1	0	2	16	5
DE	26	17	1	4†	8	31†	1	0	0	0	19	2
After 2 inoculations of *Staphylococcus aureus* culture												
C	15	7†	3	20‡	6	40†	2	1	0	0	15	3
E	18	47	14	78	15	83	6	0	1	12	14	8
DE	15	20	6	40	9	60	3	2	0	2	14	8

* Groups C were controls given bacteria without drugs; groups E received 2 subcutaneous doses of 5 mg/kg epinephrine in oil at 24-hour intervals; and groups DE received a subcutaneous dose of 50 mg/kg Dibenamine hydrochloride 24 and 48 hours before the first dose of epinephrine in oil. All rats received in random order 0.5 ml of a 5-hour bacterial broth culture intravenously 24 hr after the first dose of epinephrine in oil; the second inoculation was given 24 hr later.

† Difference from group E is statistically significant by the Chi-square test ($P < .05$).

‡ Statistically highly significant ($P < .01$).

nephrine in oil and *Staph. aureus* than in control rats given *Staph. aureus* alone. The histologic lesions were essentially similar to those induced by the same organisms in previous studies(2,3,4,5,6,7). The columns marked bacterial lesions (Table I) list the number and per cent of animals in each group with endocardial vegetations (thrombotic lesions) containing demonstrable colonies of bacteria. As shown in the columns on the right, 26 bacterial vegetations involved the mitral valve, 6 the aortic, 2 the tricuspid, none the pulmonary and 20 the mural endocardium, chiefly of the left ventricle in rats given *Staph. aureus*. The total number of bacterial vegetations exceeds the number with bacterial lesions since some rats, particularly those given epinephrine in oil, had multiple bacterial vegetations. The columns marked total endocarditis include, in addition to rats with bacterial vegetations, rats showing organizing vegetations without demonstrable bacteria and rats with a valvulitis. As shown in Table I, Dibenamine significantly reduced the incidence of endocarditis in rats given epinephrine in oil and *S. mitis*. In the rats given epinephrine in oil and *Staph aureus* the incidence of endocarditis was lower in those pretreated with Dibenamine, but the number of rats was too small to be statistically significant. However, the lesions were more severe in those not pretreated, and whereas only one rat pretreated with Dibenamine had multiple vegetations, 5 rats given epinephrine in oil without Dibenamine had multiple lesions.

Nearly all the rats given *Staph. aureus* had an acute focal interstitial nephritis (Table I) involving chiefly triangular areas of the cortex tapering into the medulla. The areas showed a dense interstitial infiltration by lymphoid cells and fewer neutrophils, a purulent exudate in some tubules, occasional tubular dilation or necrosis, and occasionally small foci of suppuration. In rats given *S. mitis*, the interstitial nephritis appeared less frequent and severe with fewer infiltrating neutrophils and suppurative foci. There was no significant difference in incidence of interstitial nephritis with either micro-organism between the controls and those given epinephrine alone or with Dibenamine. In the last column on the right in Table I are given the number of rats in each group with bacterial papillitis. This was characterized by the presence of dense bacterial masses in an area at or near the tip of a renal papilla often showing acute inflammation and necrosis (Fig. 1). The incidence of bacterial papillitis,

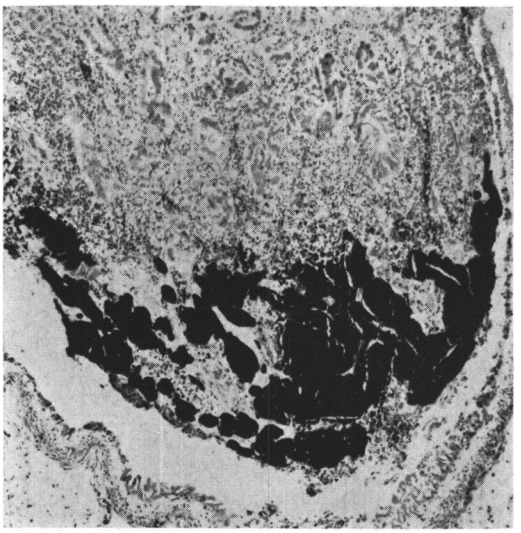

FIG. 1. Rat given epinephrine in oil and sacrificed 6 days after the first of 2 injections of *Staphylococcus aureus* showing necrosis of tip of renal papilla with darkly staining masses of staphylococci. Azure eosin (× 63).

if the 3 bacterial-treatment groups are combined, was significantly higher in the animals given epinephrine in oil alone (19 of 83 rats) than in the controls (6 of 74 rats). There was no close correlation, however between the presence or absence of interstitial nephritis or bacterial papillitis and the presence or absence of bacterial endocarditis. Some of the rats with bacterial endocarditis, however, developed renal infarcts, and bacterial emboli were occasionally demonstrable (Fig. 2).

Discussion. Our findings indicate that epinephrine in oil increases the susceptibility of rats to endocarditis induced by intravenous injection of cultures of *S. mitis* or *Staph. aureus*. This increased susceptibility is lessened or prevented by prior treatment with Dibenamine, an adrenergic blocking agent.

The cause of this increased susceptibility to endocarditis is uncertain. It is noteworthy that animals exposed to high altitude hypoxia are also markedly susceptible to bacterial endocarditis(2,3,4,5) and that the serum enzyme changes produced by altitude hypoxia are similar to those caused by epinephrine and are similarly mitigated by adrenergic blocking agents(8,9). The endocardial changes produced by high altitude(10) are also similar in many respects to those produced by epinephrine in oil(1) and, after injection of similar bacterial cultures, the lesions produced in the heart and kidney of animals exposed to high altitude are essentially similar to those found in this study. It had been postulated that the serum enzyme changes produced by epinephrine in oil may be secondary to hypoxia, a relative hypoxia due to alterations in oxidative metabolism induced by epinephrine, resulting in a markedly increased oxygen consumption particularly by the heart, and to a local hypoxia resulting from localized prolonged vasoconstriction(1). This suggests that the increased susceptibility to endocarditis may similarly be due, at least in part, to such hypoxia produced by epinephrine in oil.

There was no correlation between the presence or absence of bacterial endocarditis and the presence or absence of renal lesions other than infarcts. These findings indicate that interstitial nephritis and bacterial papillitis are not secondary to bacterial emboli derived from bacterial vegetations.

The higher incidence of bacterial papillitis in rats given epinephrine in oil than in controls, however, suggests that vascular changes induced by epinephrine in oil may play a role in the pathogenesis of this lesion. Perhaps prolonged vasoconstriction induced by epinephrine in oil may impair the circulation in the renal papilla leading to necrosis and a

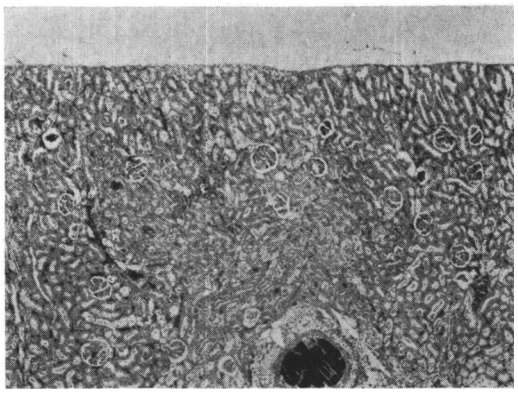

FIG. 2. Rat pretreated with Dibenamine and epinephrine in oil and sacrificed 6 days after the first of 2 injections of *Staphylococcus aureus* showing pale renal infarct above vessel containing darkly staining bacterial embolus, probably derived from a mitral vegetation that was present. Renal parenchyma on either side appears normal. Azure eosin (× 25).

lowered resistance to multiplication of bacteria.

Summary and conclusions. Five series of rats were given either 1 or 2 intravenous injections of a broth culture of *S. mitis* or 2 injections of a culture of *Staph. aureus*. Each series was divided into a control group given bacteria alone, a group given 2 daily doses of 5 mg/kg epinephrine in oil subcutaneously beginning the day before the first bacterial injection, and a third group receiving, in addition, 2 doses of 50 mg/kg Dibenamine hydrochloride 1 and 2 days before the first dose of epinephrine in oil. The animals receiving bacteria and epinephrine in oil developed a significantly higher incidence of bacterial endocarditis than either controls or rats given Dibenamine prior to epinephrine in oil. The incidence of bacterial lesions involving the renal papilla was also higher in rats given epinephrine in oil than in controls. All groups had a high incidence of focal interstitial nephritis.

It is suggested that a relative and localized hypoxia induced by epinephrine in oil may be a major factor contributing to the increased susceptibility of epinephrine-treated rats to bacterial endocarditis and renal bacterial papillitis.

The authors express their thanks to Mr. Milton G. Parker for technical assistance, to Mr. Don R. Tyson for preparing the photomicrographs, and to Dr. Paul A. Mattis and G. June Oswald of Smith, Kline and French Laboratories for a generous sample of Dibenamine hydrochloride.

1. Highman, B., Altland, P. D., Garbus, J. Arch. Path., 1965, v80, 332.

2. Highman, B., Altland, P. D., Proc. Soc. Exp. Biol. and Med., 1950, v75, 573.

3. Highman, B., Altland, P. D., Eagle, H., Am. J. Path., 1953, v29, 593.

4. Altland, P. D., Highman, B., J. Aviat. Med., 1957, v28, 253.

5. Highman, B., Altland, P. D., Eagle, H., Arch. Path., 1957, v58, 241.

6. Highman, B., Altland, P. D., Proc. Soc. Exp. Biol. and Med., 1962, v110, 663.

7. Highman, B., Roshe, J., Altland, P. D., Circulat. Res., 1956, v4, 250.

8. Highman, B., Maling, H. M., Thompson, E. C., Am. J. Physiol., 1959, v196, 436.

9. Highman, B., Altland, P. D., ibid., 1960, v199, 981.

10. ———, Arch. Path., 1949, v48, 503.

Received July 19, 1965. P.S.E.B.M., 1965, v120.

Deoxyribonuclease I and II Activities in Liver and Spleen of Mice Experimentally Infected by Mouse Hepatitis Virus.* (30665)

G. GIUSTI, L. CACCIATORE AND F. DE RITIS (Introduced by J. L. Melnick)
Infectious Disease Clinic, University of Naples, Naples, Italy

In recent years a great deal of work has been done on the metabolic changes that occur in animal tissues and in cell cultures after viral infection. We have been particularly interested in the biochemical changes in mice infected with the MHV-3 strain of murine hepatitis virus(1-3). This virus multiplies to the greatest extent in murine liver (4), but grows also in almost all other tissues including spleen, kidney, brain and myocardium(5,6). In liver, viral multiplication causes a severe necrotic hepatitis, but in other organs the damage is not very severe and is characterized by limited necrotic lesions.

Most of the biochemical changes observed during the course of experimental infection appear at the acute stage of the disease (36-48 hours after inoculation of the virus), when viral multiplication has already reached its maximum and the histological lesions are extensive. During the acute phase of the disease (48 hours after virus inoculation) many enzymatic activities are altered; an increase of phosphoglycomutase and arginase, but a decrease of adenylpyrophosphatase, succinic dehydrogenase, glutamic oxylacetic transaminase and glutamic pyruvate trans-

* Supported by C.N.R. Impresa di Virologia.