in the diagnosis of hyperthyroidism. A single blood or plasma sample of one cubic centimeter gives diagnostic values when counted after air drying. The determination of the protein-bound fraction only very rarely gives additional information but can be used to exclude spurious high levels as are occasionally found in cases of renal insufficiency where excretion of the circulating inorganic I-131 is delayed.

Received September 13, 1950. P.S.E.B.M., 1950, v75.

A New Method for the Production of Experimental Bacterial Endocarditis. (18270)

BENJAMIN HIGHMAN AND PAUL D. ALTLAND. (Introduced by Heinz Specht)

From the Experimental Biology and Medicine Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

It was shown previously(1) that discontinuous exposure of rats to simulated high altitudes of 25,000 feet may produce fibrinous vegetations and thickening of the heart valves. The vegetations, except for absence of infection, were essentially similar to those found in human bacterial endocarditis. Because of this similarity and because bacterial endocarditis in man is confined chiefly to previously damaged valves, it was considered possible that rats having heart valves damaged by exposure to altitude would be particularly suitable for the study of experimental bacterial endocarditis.

The need for further investigation of endocarditis is indicated by reports(2-5) emphasizing the difficulties of treatment, the frequent recurrences, and the relatively high mortality (about 30%). Bacterial endocarditis has been produced with varying success in animals, chiefly rabbits, by massive intravenous injections of certain strains of *Streptococcus viridans* and other microorganisms(6-8). However, since the therapeutic response of bacterial endocarditis in man often varies widely with the specific strain of the causative organism, it seemed desirable to find a more suitable laboratory animal that could be infected with greater ease and certainly with a wide variety of organisms isolated from human cases of bacterial endocarditis. As will be shown here, rats exposed to altitude seem to meet these qualifications. Moreover, our findings, indicating that resistance to disease may be lowered by exposure to high altitudes, may be of interest also in aviation medicine and to those concerned with the health of populations living at very high altitudes.

Materials and methods. In a series of 100 consecutive cases of subacute bacterial endocarditis, Niven and White(9) isolated *Streptococcus mitis* in 45 patients, *S. sanguis* in 42, *S. bovis* in 12, *S. faecalis* in 5, and various other organisms in 9 patients. In our study 5 such strains of streptococci were used. Four were isolated from cases of subacute bacterial endocarditis (*S. mitis* JH-26, *S. sanguis* P25 Type 1 and 2, and *S. bovis* P-20*, and *S. mitis* Strain N†) and the fifth was obtained from an infection of the urinary tract (*S.*

1. Highman, B., and Altland, P. D., *Arch. Path.*, 1949, v48, 503.
2. McCoy, J. T. and Meyer, O. O., *Wisc. M. J.*, 1948, v47, 671.
3. Wagner, B. W., *Am. J. M. Sc.*, 1948, v215, 84.
4. Zeller, W. W., Hirsch, H. L., Dowling, H. F., Hussey, H. H., and Robinson, J. A., *M. Ann. District of Columbia*, 1948, v17, 21.
5. Henry, L., *Postgraduate Medicine*, 1950, v7, 366.
6. Welch, H., Murdock, T. P., and Ferguson, J. A., *J. Lab. and Clin. Med.*, 1936, v21, 1264.
7. MacNeal, W. J., Spence, M. J., and Wasseen, M., PROC. SOC. EXP. BIOL. AND MED., 1939, v40, 473.
8. MacNeal, W. J., Blevins, A., Pacis, M. R. and Slavkin, A. E., *Am. J. Path.*, 1945, v21, 255.
9. Niven, C. F., and White, J. C., *J. Bact.*, 1946, v51, 790

faecalis Strain E‡). Injections of *S. faecalis* were made from 6-hour cultures in beef heart infusion broth containing 0.5% dextrose, while the other bacteria were obtained from 24-hour cultures in the broth without dextrose. Bacterial counts were made on each culture before injection by microscopic examination of a measured volume in wet coverslip preparations. The number of organisms usually ranged from 600 to 800 million per 0.5 ml, the amount injected at one time into a tail vein of each rat. Several groups of Sprague-Dawley rats were exposed 4 hours daily to a simulated altitude of 25,000 feet beginning at 21 days of age following the technique described previously(1). After 3 to 6 months (usually at about 130 days of age), these rats, henceforth designated as altitude rats, received, following their daily exposure to altitude, an intravenous injection of streptococci 4 times weekly for 3 weeks. In each experiment, a group of about 10 altitude rats and 10 non-altitude control rats of similar age and sex distribution was selected and given in random order 0.5 ml of broth from the same culture tube at each injection. Tail blood cultures were generally made 24 hours after the second, sixth, and twelfth injections (tail blood cultures of resistant animals became usually negative in 2 to 4 hours after injecting the bacteria). Autopsies were made immediately after the last blood culture or as soon as possible in case of prior death. The organs were fixed with formaldehyde, and the heart was bisected frontally after fixation. Routine paraffin sections of the tissues were stained with azure eosinate and frequently by Gram's method(10). Each half of the heart was sectioned serially. A few hearts were unsatisfactory for histologic study of the valves.

Results. As shown in Table I, 62% of the altitude rats died before completion of the series of 12 injections of streptococci and 66% developed an endocarditis; in contrast, only 9% of the control rats died and only 15% developed an endocarditis. Seven of the 8 control rats with endocarditis received injections of *S. faecalis* and 1 received 12 injections of *S. mitis* JH-26. Thirty of the 46 altitude rats with endocarditis died during the first week; only 4 of the 28 altitude rats surviving 12 injections had endocarditis. There were no significant sex differences in mortality and incidence of endocarditis.

TABLE I. Mortality and Incidence of Bacterial Endocarditis in (A) Altitude Rats and (B) Non-Altitude Control Rats Inj. with Streptococci.

Micro-organism N	No. of rats	No. died	No. hearts studied*	No. showing endocarditis
S. mitis (JH-26)				
Altitude	10	8	8	8
Control	10	0	9	1
S. mitis (strain N)				
Altitude	6	3	6	3
Control	6	0	6	0
S. sanguis (P-25 type 1 and 2)				
Altitude	21	11	19	11
Control	19	1	15	0
S. bovis (P-20)				
Altitude	11	5	11	4
Control	11	0	9	0
S. faecalis (strain E)				
Altitude	26	19	26	20
Control	20	5	14	7
Total				
Altitude	74	46	70	46
Control	66	6	53	8

* A few hearts are not included because sections of the valves were unsatisfactory for histologic study.

Endocarditis could be predicted antemortem in nearly all altitude rats by the development of a positive blood culture associated with progressive loss in weight, usually ranging from 1 to 5 g per day. The loss in weight was usually greater in males and in the 48 hours preceding death. A few rats with endocarditis lost weight but had negative cultures. Studies in progress indicate that in such cases a second culture taken after a 24-hour rest period may be positive. A positive blood culture with slight loss in weight was

* Furnished by Dr. J. M. Sherman, Cornell University, Ithaca, N. Y.

† Furnished by Dr. C. H. Binford, U. S. Marine Hospital, Baltimore, Md.

‡ Furnished by Dr. H. Eagle, National Institutes of Health, Bethesda, Md.

10. Lillie, R. D., Histopathologic Technic, Philadelphia, Pa., The Blakiston Company, 1948.

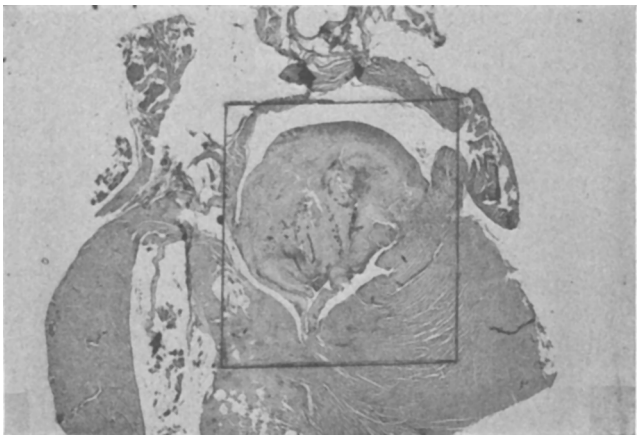

FIG. 1.
Photomicrograph of bacterial vegetation on mitral valve of rat dying after 2 intravenous injections of *Streptococcus mitis* (strain N). Bacterial colonies are dark and lie at several levels in the vegetation. Azure eosinate. Magnification ×5.

seen in some control rats without endocarditis; these animals often showed severe renal changes, an extensive pneumonia, or abscesses in the spleen or other organs.

Rectal temperatures were taken hourly on several occasions, with some animals showing a rise of 1 to 2°C at 2 to 6 hours after an injection. This rise was not considered to be of great diagnostic value because of the wide fluctuations in temperature encountered in many uninjected altitude rats and in some non-altitude rats. A subnormal temperature and great listlessness were noted in some rats for a few hours preceding death. Leucocyte counts taken after a series of daily injections of *S. faecalis* in one experiment ranged between 15,000 and 25,000 in both altitude and nonaltitude rats. Streptococci were found in cultures of the urine aspirated at autopsy from the urinary bladder of a few animals showing focal renal lesions.

Bacterial vegetations were found on about 54% of the mitral valves observed and on 31% of the aortic valves, and in some cases they were present on both valves or on the mural endocardium of the left atrium. No vegetations were found on the tricuspid or pulmonary valves in the animals included in this report. The lesions tended to be most frequent and severe along the line of closure, but often involved other areas and sometimes nearly the entire surface of one or more valve leaflets. Sections of the involved leaflets showed edema, cellular infiltration and marked fibrous thickening with fibroblasts often accompanied by round cells and polymorphonuclear leucocytes extending into the base of the vegetation. The bacterial vegetations were composed chiefly of fibrin often admixed basally with necrotic debris and more superficially with polymorphonuclear leucocytes and red blood cells. Large discrete and confluent colonies of streptococci were found at one or more levels in the superficial and mid-portions of the vegetations (Fig. 1). There were usually some colonies along the surface, but occasionally all seemed covered by a thick layer of fibrin, perhaps accounting for the occasionally negative blood cultures reported above. A few isolated bacteria, often admixed with leucocytes and necrotic debris, were sometimes found in other portions of the vegetations and occasionally in the fibrous tissue at the base of the vegetations. Polymorphonuclear leucocytes were found in variable numbers chiefly at the base and in the superficial portion of the vegetations, and only occasionally near the large bacterial colonies (Fig. 2). The leucocytes were often fragmented and seldom contained bacteria.

In another study(1), 28 rats of corre-

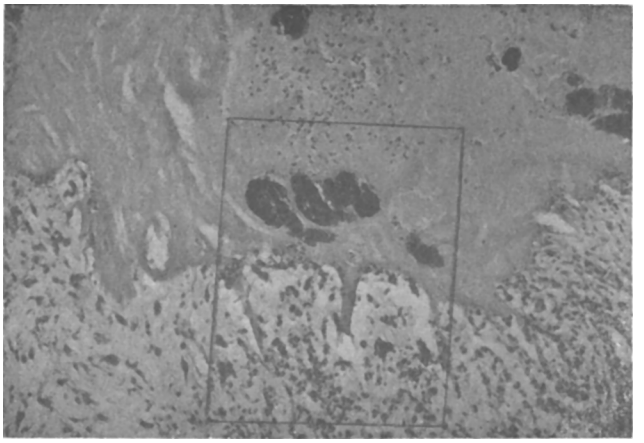

FIG. 2.
Photomicrograph from area at base of vegetation shown in Fig. 1. Azure eosinate. Magnification ×100.

sponding age were exposed to altitude but did not receive streptococci. Nearly all showed thickening and other degenerative changes in the valves, but only 10 (36%) had vegetations (sterile). In the present study, the incidence of bacterial vegetations after injection of streptococci was much higher (66%). This suggests that many bacterial vegetations were formed on damaged valves without antecedent sterile vegetations. It is of interest that several injected altitude animals showed sterile valvular vegetations in areas distant from bacterial vegetations, and 1 animal killed for study following 12 injections of *S. bovis* showed a sterile vegetation on the aortic valve without evidence of infection anywhere.

In many animals there was a severe cellular reaction in the myocardium near the bacterial vegetations, frequently extending to adjoining valves and to the epicardium and adventitia of the large blood vessels. Many of the muscle fibers near the vegetation showed indistinct cross-striations, atrophy, or necrosis; the interstitial tissue showed marked proliferation of fibroblasts and a dense infiltration by small and large mononuclear cells and variable numbers of polymorphonuclear leucocytes and cells with rod-shaped nucleoli as found in *Anitschkow myocytes*. No typical Aschoff bodies were found. The hearts of a few rats showed similar but smaller inflammatory foci in the myocardium distant from the valves. Small hemorrhages were seen occasionally in some of the infected valves and in the epicardium near such valves. The heart of 1 rat dying after 4 injections of *S. mitis* showed a bacterial embolus in a branch of the coronary artery with a perivascular infiltration by polymorphonuclear leucocytes.

The kidneys of many altitude rats, chiefly those that died during the first 4 days after the initial injection, showed hyaline droplet formation, epithelial desquamation or necrosis involving many cortical tubules. These changes were similar to but usually more severe than those seen in some altitude rats not receiving streptococci. A few such kidneys showed erythrocytes or streptococci in the lumens of occasional tubules.

Focal renal lesions were encountered after the fourth day in nearly all animals injected with *S. mitis* JH-26 and *S. faecalis*, but in none injected with the other organisms. There were small areas in the cortex infiltrated chiefly by lymphocytes and showing dilated tubules containing cellular debris, a purulent exudate and sometimes bacteria. In addition there were usually a few necrotic tubules in such areas and rarely a necrotic glomerulus. Similar lesions were seen at times in the medulla, and occasionally a purulent exudate and bacteria were found in the renal pelvis.

One rat that died after 4 injections of *S. faecalis* showed bacterial emboli in many glomerular and intertubular capillaries throughout both kidneys.

Three injected animals had old calcifying sterile renal infarcts (typical of altitude exposure), and 1 had a recent infarct with a markedly congested border densely infiltrated by polymorphonuclear leucocytes. There were many marginal micro-abscesses and scattered clumps of streptococci, some extending into the infarcted area.

The lungs of many rats that died showed intense congestion and often extensive edema, hemorrhages or pneumonia. Several showed centrolobular necrosis in the liver and a few had multiple abscesses chiefly in the lungs or spleen.

Summary and conclusions. Our findings indicate that bacterial endocarditis may be induced readily in rats exposed 4 hours daily to simulated high altitude (25,000 ft.). Twenty-six of 44 altitude rats, but only 1 of 39 non-altitude controls, developed bacterial endocarditis after intravenous injections of *S. mitis, S. sanguis* and *S. bovis* obtained from human cases of subacute bacterial endocarditis. Endocarditis was found in 20 of 26 altitude rats and in 7 of 14 non-altitude controls injected with *S. faecalis*. The incidence of endocarditis and the character of the lesions in other organs varied with the species of streptococcus used. It is suggested that altitude rats may be used to study the reaction and to test the effectiveness *in vivo* of various therapeutic measures against specific strains of organisms causing bacterial endocarditis in man. It is also suggested that exposure to altitude may be a useful experimental method for rendering resistant species of animals susceptible to some diseases other than endocarditis.

The authors wish to express appreciation to Dr. Roy Schneiter for valuable suggestions during the course of this study, and to John Simpson for technical aid.

Received August 4, 1950. P.S.E.B.M., 1950, v75.

A Fermentable Non-Reducing Substance in Plasma Following Insulin Administration.* (18271)

ARNE N. WICK, DOUGLAS R. DRURY AND EATON M. MACKAY. (With the technical assistance of Richard W. Bancroft, Robert Fitch, Nancy Hillyard, Mary E. Blackwell and Milford E. Dorough)

From the Scripps Metabolic Clinic, La Jolla, Calif., and the Department of Physiology, University of Southern California, Los Angeles.

During the course of a study of the action of insulin on glucose oxidation(1) it was found that insulin administration leads to the appearance of a fermentable (*i.e.* liberates CO_2 under the action of yeast) non-reducing substance in the plasma of rabbits. This report concerns the conditions under which this substance is formed.

Experimental. The general plan of the experiments was as follows. After evisceration(2) the rabbits were injected with a priming dose of a potent preparation of uniformly labeled carbon 14 radioactive glucose. They were then maintained for 8 hours with a normal blood sugar level by the constant injection of a radioactive glucose solution calculated to have the same specific activity as that of the circulating plasma glucose resulting from the priming dose. Two groups of eviscerated animals were used and treated

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

1. Wick, A. N., Drury, D. R., Bancroft, R. W. and MacKay, E. M., *J. Biol. Chem.*, Dec., 1950.

2. Drury, D. R., *Am. J. Physiol.*, 1935, v111, 289.