TABLE I. η_{sp}/Φ of A-Substance.

$\Phi \cdot 10^3$	Average shear gradient of solvent 10200	5400	1000
R19-F1A			
3.91	150	210	300
1.96	130	170	260
0.78	—	140	240
R19-F2A			
3.31	190	250	400
1.65	170	220	330
0.66	170	220	290

Viscosity. The values of specific viscosity, η_{sp}, divided by volume fraction of solute, Φ, at different rates of shear are given in Table I. Measurements were made in an Ostwald-Fenske viscometer at 25°C.

Osmotic pressure. Four measurements at one and 2% concentration gave the molecular weight of R18-F10 as 220,000 ± 30,000, uncorrected for deviations from ideal behavior.

Discussion. If the molecules are assumed to be anhydrous rod-like ellipsoids, the viscosity data lead to a value of 60/1 for the axial ratio. This value, together with diffusion and density data, gives an average value of 1,000,000 for the molecular weight.

Table I shows the marked dependence of the value of η_{sp}/Φ on shear gradient and on concentration. This behavior is more like that of a rod-like particle than a random coil.§

The molecular weights from light scattering data agree fairly well with that from viscosity and diffusion. Osmotic pressure data lead to a much lower value. Since the latter method gives a number-average molecular weight and the light-scattering method gives a weight-average molecular weight, the results are compatible with the assumption that the material is heterogeneous. The data do not permit one to decide whether a few per cent of larger or smaller molecules account for the results, or the preparations contain a broad distribution of sizes with significant numbers of molecules with molecular weights less than 200,000 and greater than one million.

It may be *concluded* that A-substance from hog gastric mucin, like A-substance from pseudomucinous ovarian cyst fluid, is a very large molecule and probably is highly asymmetric.

§ We are indebted to Dr. Hans Kuhn for interpreting these data.

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Experimental Bacterial Endocarditis in Altitude Rats II. Shortening Acclimatization Period. (19150)

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The mortality rate of subacute bacterial endocarditis, despite antibiotic therapy, averages about 30%(1). Lack of a suitable experimental animal has handicapped hitherto the search for more effective treatment. The screening of therapeutic methods calls for a readily available small laboratory test animal which is highly susceptible to experimentally induced bacterial endocarditis. Since the therapeutic response in man often varies considerably with the specific infectious agent, the experimental animal should be susceptible to various strains obtained from human cases of bacterial endocarditis.

It was shown previously that exposure of rats 4 hours daily for 3 to 6 months in a low pressure chamber to simulated altitudes of 25,000 feet (7620 meters, bar. p. 282 mm Hg) damages the cardiac valves(2), and that ex-

1. Henry, L., *Postgrad. Med.*, 1950, v7, 366.

2. Highman, B., and Altland, P. D., *Arch. Path.*, 1949, v48, 503.

TABLE I. Mortality Rate, Hematocrit Values, and Incidence of Bacterial Endocarditis in Rats given 8 Intravenous Injections of *Streptococcus mitis*, JH-26, in 12 Days. The Rats Were Exposed to 25000 Feet for Varying Periods before Beginning the Injections at Age 130 Days.

Group	Altitude exposure before inj. (days)	No. of rats	No. died	Mean hematocrit value†		Endocarditis	
				Before inj.	After inj.	No.	%
1.	30	23	12	78.0 ± 1.5	73.4 ± 1.4‡	20	87
2.	30*	16	4		57.8 ± 2.8	7	44
3.	20	16	9	76.5 ± 1.4	71.2 ± 1.1‡	11	69
4.	10	17	4	65.2 ± 1.8	65.8 ± 2.5	8	47
5.	0	11	4		55.9 ± 1.9	5	45
6.	0*	24	7		42.4 ± 0.7	4	17

* Groups 2 and 6 not exposed to altitude after beginning bacterial inj.

† Numbers indicate mean hematocrit values ± the stand. error of the mean. Hematocrit determinations made using 4 to 10 animals per group, immediately before beginning injections and again immediately before autopsy.

‡ Drop in mean hematocrit value after injections is statistically significant. Since group 4 was exposed to altitude during the injection period, the hematocrit value, had no injections been given, should have exceeded 76.5.

perimental bacterial endocarditis could be readily induced in such altitude-exposed rats by the intravenous injection of streptococci (3). Because of the remarkably high incidence of endocarditis produced in such rats with various strains of streptococci isolated from human cases of subacute bacterial endocarditis, and because the rat is particularly suitable for large scale laboratory testing, the method was considered valuable for studies aimed at improving the treatment of bacterial endocarditis. To facilitate such studies, however, an effort was made to reduce the number of bacterial injections and to shorten the period of acclimatization to altitude, and thereby reduce the labor entailed in exposing animals to altitudes over a long period. In this paper we shall show that a high incidence of endocarditis may be induced in altitude rats even when the acclimatization period is less than 30 days. An attempt was also made to correlate the susceptibility to endocarditis with the severity of the polycythemia.

Materials and methods. The methods used in exposing the animals to altitude and in preparing and injecting the bacteria were essentially similar to those described previously(2,3). A series of Sprague-Dawley rats was maintained at ground level (about 340 feet, 104 meters) until 100 days of age and then divided into groups of 11 to 24 animals each. The exposures to altitude (25,000 feet) were begun at staggered intervals so that at 130 days of age, when the first bacterial injection was given, one group each had been exposed 10 and 20 days and 2 groups each 0 and 30 days. During the following 12 days the rats in all groups received 8 intravenous injections, each consisting of 0.5 cc (500 to 800 million organisms) of a 6-hour culture of *Streptococcus mitis* JH-26 in beef heart infusion broth containing 0.5% dextrose. This organism was originally isolated from a case of subacute bacterial endocarditis and was selected because in the previous study nonaltitude rats were found to be relatively resistant to this streptococcus. Survivors were killed 3 days after the last injection. Only one of each of the 0-day and 30-day groups was exposed to altitude during the injection period. Hematocrit values were determined with Van Allen tubes on 4 to 10 animals per group, and endocarditis was determined by histologic studies as previously described(3).

Results. As shown in Table I, the incidence of endocarditis seems to vary with the mean hematocrit value. The mean hematocrit value after 30 days acclimatization was 78 or approximately the maximum attainable by rats exposed to 25,000 feet(2). The incidence of endocarditis in the 23 rats was 87% after 8 injections compared with 100% reported previously after 12 injections in 8 rats exposed

3. Highman, B., and Altland, P. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 573.

to altitude for 3 to 6 months. In the 30-day group the mean hematocrit value dropped to 73.3 (statistically significant) after the injections, probably due to the infection. When the exposures to altitude were discontinued during the injection period, however, the drop in hematocrit value was much greater and the incidence of endocarditis was approximately halved. The incidence of endocarditis in other groups likewise seemed to vary with the mean hematocrit value.

As in the previous study, the animals that developed an endocarditis generally lost considerable weight and nearly all rats that died or were killed after the fourth day from the first injection had renal abscesses. Some showed large bacterial masses in the renal pelvis and extensive necrosis of the renal papillae. Endocarditis was found in nearly all the altitude rats that died including 9 of 10 in the 20-day and 30-day groups that died after only 4 injections. None in these groups died after less than 4 injections. In the previous study, however, endocarditis was found in some animals that died after only a single injection of *S. mitis* or other strains of streptococci, particularly *S. faecalis* Strain E.

To study the incidence of endocarditis after a single injection, therefore, 15 altitude rats (exposed 70 days to 25,000 feet) were given a single intravenous injection of 0.5 cc (800 million organisms) of a 6-hour culture of *S. faecalis* Strain E in beef heart infusion broth containing 0.5% dextrose. Bacterial vegetations were found on the heart valves in 3 of 6 such rats killed 20 hours after the injection, in 2 of 3 killed at 48 hours, and in 3 of 3 each killed at 72 and 96 hours after the injection. Two of the 4 animals without bacterial vegetations showed inflammatory changes in the mitral valves, suggesting that vegetations might have ensued if the animals had not been killed. Although this incidence following a single injection is high, it must be emphasized that the incidence after multiple injections was found to vary considerably with different species of streptococci and to a lesser extent in different groups of animals.

Discussion. Since susceptibility to endocarditis seems to vary with the hematocrit value, it is possible that the acclimatization period might be further shortened by prolonging the daily exposures to altitude to speed the rise in hematocrit value. It is also possible that susceptibility might be induced by ground level hypoxemia or by other methods increasing blood volume(4).

The polycythemia induced by altitude increases the work load on the heart and its valves. That this may be an important factor in increasing the susceptibility to endocarditis is suggested by the work of Lillehei, Bobb and Visscher(4). They reported an increased susceptibility to endocarditis in dogs in which an increase in work load on the heart was accomplished by the creation of large arteriovenous fistulae. That additional factors may be involved, however, is indicated by evidence that exposure to high altitude may lower the resistance to other types of infection. For example, it has been shown recently(5), that CF-1 mice made polycythemic by exposure to high altitude were more susceptible to artificially induced *Salmonella typhimurium* infections than normal or anemic mice.

Summary and conclusion. Sprague-Dawley rats were exposed in a low pressure chamber 4 hours daily 0 to 30 days to a simulated altitude of 25,000 feet (bar. p. 282 mm Hg). During the following 12 days, they were given 8 intravenous injections of *S. mitis*. Bacterial endocarditis was found in 87% of the rats acclimatized 20 days, but in only 44% if the exposures to altitude were discontinued after the first injection. It was found in 17% of rats never exposed to altitude, and in 45% of those beginning exposures to altitude immediately before their first injection. Endocarditis was found in 11 of 15 altitude rats given a single injection of *S. faecalis*. The susceptibility to endocarditis in this study varied with the mean hematocrit value. Since the hematocrit value approaches a maximum in about 30 days, there seems to be little indica-

4. Lillehei, C. W., Bobb, J. R. R., and Visscher, M. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 9.

5. Aero Medical Subcommittee. USAF School of Aviation Medicine and Arctic Aeromedical Laboratory Supplement. Air Force Semi-Annual Progress Report, 1951, v2, Proj. No. 21-35-005.

tion for increasing the preliminary exposures to altitude beyond 30 days. It is suggested that such altitude rats be used for screening of methods for the treatment of bacterial endocarditis in man.

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Endogenous O_2 Uptake of Intact Embryos, Homogenates and Cellular Constituents in Isotonic Sucrose and Ringer.* (19151)

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In the preparation of homogenates from vertebrate tissues, and especially liver, it has been shown that sucrose makes a more satisfactory suspension medium than isotonic balanced salt solution(1-3). This is especially true when the activity of certain enzyme systems is being studied, for example, succinoxidase. Greater activity has been obtained when homogenates and cellular constituents are suspended in isotonic sucrose. For in-

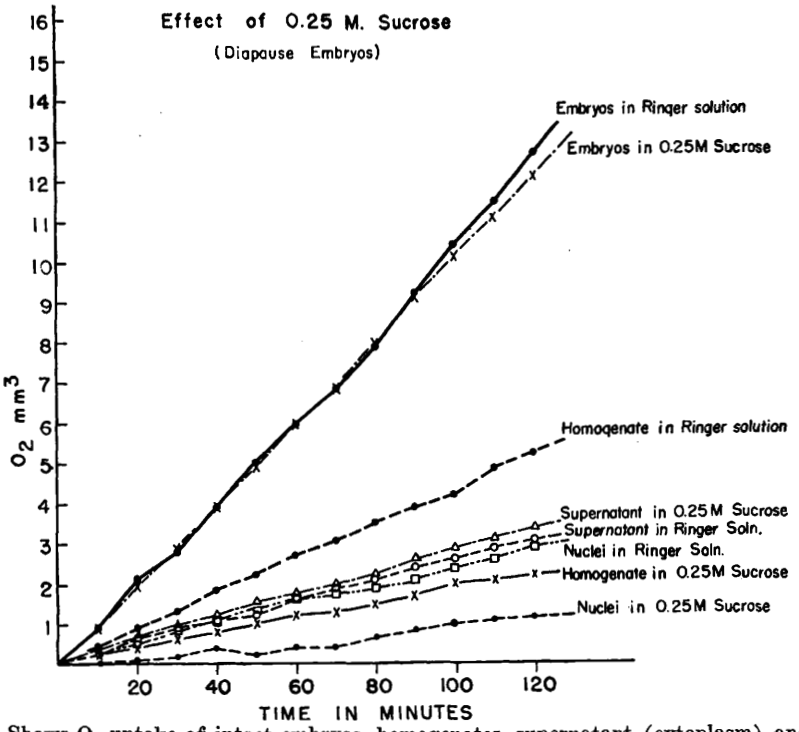

FIG. 1. Shows O_2 uptake of intact embryos, homogenates, supernatant (cytoplasm) and intact nuclei in .25 M sucrose and Ringer's solution. Arrow indicates time of emptying contents of side arm into respiration flask.

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1. Schneider, W. C., Claude, A., and Hogeboom, G. H., *J. Biol. Chem.*, 1948, v172, 451.

2. Lardy, H. A., *Respiratory Enzymes*, Burgess Publ. Co., Minneapolis, 1949, (Contains rather complete bibliography).

3. Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 320.