range. Lipid concentration, however, is nearly constant in tissue weighing more than 1.0 g. Rather large changes in $W_T$ may occur with little effect on the value of $W_L/W_T$ in the area of the plateau in Fig. 2. The opposite is true in tissue below 1.0 g, where differences of lesser magnitude in $W_T$ reflect large differences in $W_L/W_T$.

If one were investigating nutritional, drug or hormone effects on epididymal adipose tissue, tissue weight or lipid content would reflect the treatment effect best above 1.0 g; below this value, percentage lipid would be a more sensitive measure.

Transformation of the data of this study to the form shown in Fig. 3 concisely illustrates the inter-relationships between lipid, water and residue concentration in the epididymal fat. However, it must be reemphasized that presentation in this form compresses the area of Fig. 2 where $W_L/W_T$ is quite constant for a wide range of $W_T$ values and tends to expand the changes in $W_L/W_T$ for small differences in $W_T$ values.

Residue and water appear to be present in epididymal fat tissue in constant amounts. Thus the concentrations of these constituents decrease with increasing adipose tissue fresh weight. Similar characteristics have been observed for perirenal adipose tissue of the rat (7).

*Summary.* A high positive correlation ($r = 0.9943$) was observed between fresh weight and lipid content of epididymal adipose tissue weighing more than about 0.5-1.0 g. Above this value lipid concentration in the tissue was 80-85% and was relatively constant over a wide range of weights. These relations suggest that for tissues weighing more than 0.5-1.0 g the weight or lipid content of the tissue is the most sensitive measure of treatment effects; lipid concentration is more sensitive below these values.

We gratefully acknowledge the expert analytical assistance of Messrs. J. Perrine and W. Pierce and statistical analyses by Mr. R. Lamm.

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Received May 29, 1962. P.S.E.B.M.. 1962. v110.

## Effect of Exposure and Acclimatization to Cold on Susceptibility of Rats To Bacterial Endocarditis. (27610)

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The influence of exposure to cold on susceptibility to disease has been of concern for many years(1). Interest in this field has been renewed with the increasing use of hypothermia, particularly in surgery, and with increasing concern with the colder regions of the earth, outer space, and factors involved in acclimatization to cold. Previous studies have yielded conflicting results, which varied with the disease and the experimental conditions, and point to the need for further clarification.

Angrist, Oka, Nakao, and Marquiss(2) reported that rats given repeated 4-hour exposures to 4°C developed changes in the cardiac valves similar to those found in rats exposed repeatedly to simulated high altitude (3). Since we had found that bacterial endocarditis can be readily induced in rats exposed to high altitude(4,5), the present study was undertaken to determine if rats exposed

to cold, either acutely or after acclimatization, showed a similar increased susceptibility to endocarditis. We selected a cold environment (1.7°C) which was tolerated by rats and which, we had found, did not cause significant hypothermia, but produced a rise in serum enzyme levels after an acute exposure(6) similar to that produced by simulated high altitude (7).

*Methods.* Several series of young adult male Sprague-Dawley rats, 90-110 days of age and 250-350 g in weight, were selected. Each lot was divided into a control group maintained at normal room temperature and one or more experimental groups kept for varying periods in a cold room maintained at 1.7 ± 0.6°C, as previously described(6). The rats were housed singly in metal cages and given a Purina Laboratory Chow diet and water *ad libitum*. The rats exposed to cold and their corresponding controls were given in random order an injection in the tail vein of 0.5 ml of a 5-hour broth culture of *Streptococcus mitis* JH 26, as previously described (4). In most experiments, the rats received only one bacterial injection, while in some early experiments they received an injection on 4 successive days (Table I). Duration of cold exposure before and after the bacterial injections was varied in different experiments (Table I). Survivors were killed 7 days after the first or single bacterial injection. Autopsies were performed on all rats that died or were killed after receiving bacteria, and the heart, lung and kidney were fixed in a 10% aqueous solution of buffered (pH 7.0) formalin. The heart was bisected frontally after fixation 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 lung and kidney were stained with azure eosin.

*Results.* The incidence of bacterial vegetations was much higher in rats kept in the cold room after intravenous injection of streptococci (Table I). No significant difference in incidence was found between the groups receiving 1 and 4 bacterial injections and between those kept in the cold room 1, 14 and 35-36 days before bacterial injection. The incidence of bacterial endocarditis in rats kept in the cold room 36 days before a bacterial inoculation and then maintained at normal room temperature was similar to that of the controls. In rats kept in the cold room beginning immediately after a bacterial inoculation, the incidence of endocarditis was intermediate between that of the controls and those maintained in the cold room beginning 1-36 days before and up to 7 days after inoculation. This suggests that the increase in susceptibility does not reach a maximum until at least several hours after beginning the cold exposure.

In rats kept in the cold room following bacterial inoculation, the mortality rate was lowest in the groups acclimatized 35-36 days and highest in the 1-day group (Table I). In the

TABLE I. Effect of Exposure to Cold (1.7°C) on Mortality and Incidence of Endocarditis in Rats Given 1 or 4 Intravenous Injections of *Streptococcus mitis* JH 26.

| Days in cold (1.7°C) | | | Endocarditis | | | | Deaths | | No. with bacterial vegetations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Severe | | Total | | | | | | |
| No. of rats | Before inj. | After inj. | No. | % | No. | % | Rate, % | Mean day | Mitral | Aortic | Other |
| After 4 I.V. injections of *S. mitis* | | | | | | | | | | | |
| 19* | 0 | 0 | 3 | 16 | 6 | 32 | 5 | 6.0 | 3 | 0 | 0 |
| 10 | 14 | 7 | 10 | 100 | 10 | 100 | 70 | 5.9 | 10 | 3 | 1 |
| 22 | 35 | 7 | 18 | 82 | 19 | 85 | 41 | 5.7 | 15 | 8 | 6 |
| After 1 I.V. injection of *S. mitis* | | | | | | | | | | | |
| 16* | 0 | 0 | 3 | 19 | 3 | 19 | 0 | 0 | 3 | 0 | 0 |
| 14 | 0 | 7 | 7 | 50 | 11 | 79 | 43 | 6.0 | 6 | 1 | 2 |
| 15 | 1 | 7 | 13 | 87 | 13 | 87 | 73 | 4.2 | 12 | 2 | 5 |
| 10 | 14 | 7 | 9 | 90 | 9 | 90 | 70 | 5.4 | 8 | 2 | 2 |
| 11 | 36 | 7 | 9 | 82 | 11 | 100 | 55 | 5.9 | 9 | 4 | 2 |
| 11 | 36 | 0 | 2 | 18 | 5 | 45 | 9 | 7.0 | 1 | 1 | 0 |

* Control groups kept at room temperature throughout experiment.

latter group, 11 of the 15 rats died with a mean length of survival of 4.2 days. All of these 11 rats died in 4 or 5 days, whereas 20 of 30 rats that died in the groups acclimatized 14-36 days survived more than 5 days; this is statistically significant by the chi-square test ($P<.01$).

The vegetations were histologically similar to those previously induced by *S. mitis* in rats exposed to simulated high altitude(4,5,8) and were comparably graded(8). In 74 rats (Table I), endocarditis was classified as severe, since bacteria were readily demonstrable in one or more vegetations on the valves or mural endocardium. In 25 of the 74 rats, additional nonbacterial lesions were found in other portions of the heart. Both bacterial and nonbacterial lesions affected chiefly the mitral and aortic leaflets and were most frequent and severe in the anterior (aortic) mitral leaflet. However, in 7, 1, 4, 4, and 2 rats, bacterial lesions were confined to the tricuspid valve, aortic intima, left ventricle, left auricle and right ventricle, respectively. In addition to the 74 rats with bacterial vegetations, the total columns in Table I include 13 additional rats with less severe endocardial lesions showing no demonstrable bacteria; 4 of the 13 rats had a mitral valvulitis, and 9 had organizing vegetations, indicating regressing lesions(8).

About one-fourth of the rats with bacterial vegetations had bacterial lesions, presumably embolic, in branches of the coronary arteries; about half had small myocardial infarcts; and nearly all had a severe focal and perivalvular myocarditis. Control rats and rats maintained at room temperature after the bacterial injection showed no coronary bacterial lesions and no myocardial infarcts, and myocarditis was less frequent and severe in such rats.

Nearly all the rats with bacterial vegetations had one or more types of severe renal lesions, such as areas of acute interstitial nephritis, foci of suppuration, septic infarcts, septic emboli, and inflammatory foci in the renal papilla, some papillae containing masses of bacteria. Such renal lesions were less frequent and severe in control rats.

Pulmonary edema, hemorrhages, and interstitial pneumonitis, usually mild, were seen in 17, 26, and 20% of the rats respectively; there was no significant difference in incidence and severity of these pulmonary lesions between the controls and the other groups.

Twelve rats which were not given bacteria had rectal temperatures taken daily. Their mean temperature before they were placed in the cold room was 38.0°C, and during a period of 16 days in the cold room ranged between 37.2 and 37.9°C.

*Discussion.* Our results indicate the following: Continued exposure to cold (1.7°C) increases the susceptibility of rats to endocarditis induced by an intravenous injection of a culture of *S. mitis*. Placing the rats in the cold room one day before the bacterial inoculation is sufficient to produce marked susceptibility. Keeping the rats in the cold room after bacterial inoculation is necessary to maintain their susceptibility. Acclimatization to cold, by placing rats in the cold room 14-36 days before inoculation, does not significantly alter the incidence of endocarditis in comparison with rats placed in the cold room one day before inoculation but does appear to prolong the life of rats with severe infections.

Previous studies on the effects of chilling and cold exposures have led to conflicting results and interpretations. While some workers reported no increased susceptibility or even increased resistance to infection induced by cold(1,9), most investigators reported an increase in susceptibility(1,10). In general, however, the studies of earlier investigators, reviewed by Perla and Marmorston(1), and of more recent workers(9,10) differed from our studies in that they were not concerned with endocarditis, their cold exposures were more severe but relatively brief, and their animals were rendered hypothermic.

There seem to be many similarities between the effects of exposure to cold and exposure to high altitude(11). Both cause hypertrophy of the adrenal gland(3,11). Prolonged exposure to either cold or simulated high altitude produces similar histologic changes in the cardiac valves(2,3). Both environments cause a comparable increase in susceptibility to bacterial endocarditis(4,5,8,12). Since it was shown(12) that high altitude rats are much more susceptible to endocarditis than

ground level rats given large doses of cortisone, it seems unlikely that the increased susceptibility can be attributed solely to hyperactivity of the adrenal cortex. Similarly, this increased susceptibility in both environments is probably not due to impaired antibody formation. Thus, high altitude rats injected with sheep erythrocytes formed increased amounts of antisheep erythrocyte hemolysin during the primary immune response without change in length of the induction period compared to controls(13). More recently, Trapani and Jordan(14) reported that circulating precipitins reached higher levels after an intravenous injection of bovine serum albumin in rabbits adapted to high altitude than in control rabbits. Similarly, Goodman, Neidengard and Heiskell(15) used gamma-globulin, Cohn fraction II, in adjuvant as an antigen in mice and found that antibody levels were greater in hypothermic mice than in mice kept in a normal temperature environment.

There is some evidence that exposure to cold does not reduce phagocytic activity. Thus, Fedor, Fisher and Fisher(9) reported in dogs subjected to hypothermia and subsequent rewarming that leukopenia is transitory and is followed by a leukocytosis resulting from a marked increase in band cell neutrophils, that no reduction in phagocytosis of blood leukocytes was evident during hypothermia or following rewarming, and that the electrophoretic patterns of plasma demonstrated no change in phagocytic promotion factors. It is noteworthy that we found similar changes in total and differential white cell counts in rats exposed 16 hours to 1.7°C even in the absence of significant hypothermia(6). On the other hand, Gowen and Friou(10) reported that in the hypothermic dog the blood stream is not cleared of injected viable bacteria as quickly as in a normothermic dog. We found that high altitude rats showed a similar delay in comparison with ground level controls (unpublished data). These and other findings(8) suggest that, while phagocytic activity may not be reduced, the ability of phagocytes to destroy bacteria may be impaired.

An acute exposure to either cold, even without hypothermia(6), or high altitude(7) is followed by a sharp rise in certain serum enzymes such as aldolase and glutamic oxalacetic transaminase. Changes in levels of various tissue enzymes and other biochemical changes(11,16,17) have also been reported after such exposures. Accordingly, since the evidence presented above suggests no impairment in phagocytic activity or antibody formation, it is postulated that some of the enzymatic and biochemical changes induced by cold impair the ability of the host phagocytic cells to destroy specific bacteria and that such changes may contribute to the increased susceptibility to bacterial endocarditis induced by cold exposure.

*Summary.* Groups of rats were exposed in a cold room maintained at 1.7°C for periods ranging from 0 to 36 days and showed little or no reduction in body temperature during this period. These groups, along with appropriate controls maintained in a normal temperature environment, were then given either 1 or 4 intravenous injections of a broth culture of *Streptococcus mitis* JH 26. Survivors were killed for pathologic study 7 days after the first bacterial inoculation. In control groups and in the one group of cold rats returned to a normal temperature environment after the bacterial inoculation, the incidence of severe bacterial endocarditis was 16-19% and mortality rate was 0-9%. In the groups kept in the cold room 1-36 days before and 7 days after the first inoculation, the incidence of severe bacterial endocarditis was 82-100% and the mortality rate 41-73%. The corresponding figures for rats kept in the cold room after but not before inoculation were 50 and 43% respectively. In rats acclimatized to cold for 35-36 days, the mortality rate was lower and length of survival was longer than in rats placed in the cold room only one day before bacterial inoculation.

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Received May 31, 1962. P.S.E.B.M., 1962, v110.

## A Transduction Analysis of Complex Loci Governing the Synthesis of Tryptophan by *Staphylococcus aureus.** (27611)

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Transduction analysis of complex loci involved in the synthesis of tryptophan(1), adenine(2), histidine(3), and cysteine(4), in *Salmonella typhimurium* and tryptophan(5) and arabinose(6) in *E. coli* have been described. In each instance auxotrophic mutants lacking the ability to synthesize a given growth factor were divided into functional groups which correspond to the arrangement of a group of mutant sites on the chromosome. The order of sites on the chromosome was the same as the sequence of biochemical reactions that the sites controlled. Transduction technics make possible the genetic mapping of loci involved in the synthesis of a metabolite by strains of *S. aureus*(7,8,9,10). This report concerns an analysis of the genetic relationship among tryptophan-dependent mutants of *S. aureus*.

*Methods.* The strain of *S. aureus* employed, W-26, originally obtained from The Ohio State University Hospital, and the mutants derived from it, were maintained on brainheart infusion (BHI) agar slants at 4°C.

The minimal medium (M) contained Casamino acids, acid-hydrolyzed (Difco)—500 mg; l-cystine—10 mg; dextrose—1000 mg; sodium citrate $5H_2O$—500 mg; $K_2HPO_4$—500 mg; $MgSO_4$—10 mg; NaCl—4 mg; $FeSO_4 \cdot H_2O$—4 mg; $MnCl_2 \cdot 4H_2O$—15 mg; adenine sulfate—1 mg; guanine hydrochloride—1 mg; uracil—1 mg; xanthine—1 mg; thiamine hydrochloride—0.1 mg; nicotinic acid—0.5 mg; and distilled water to make 100 ml. For a solid medium 1.5% washed agar was added, citrate was omitted, and the dextrose was added aseptically after autoclaving. Both broth and solid media had a pH of 6.6 to 6.8 after autoclaving. When desired, 5 mg of tryptophan, 10 mg of anthranilic acid, or 10 mg of indole were added to 100 ml of M medium to make MT, MA, or MI medium, respectively.

Tryptophan-requiring mutants were selected by the penicillin screening method of Davis(11) and Lederberg(12) from a suspension of ultra-violet irradiated *S. aureus* W-26. One ml of the irradiated suspension was inoculated into 5 ml of MT broth and incubated at 37°C for 24 hrs; the culture was centrifuged and resuspended in 5 ml of double distilled deionized water. One ml of this suspension was then added to 4 ml of M broth containing 1.25 units of penicillin per ml.

* Presented in part as partial fulfillment of the degree Doctor of Philosophy at The Ohio State University. Supported by a grant from Nat. Science Foundation.

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