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EFFECT OF ACCLIMATIZATION TO 2° C ON HOST SUSCEPTIBILITY TO KLEBSIELLA PNEUMONIAE CHALLENGE

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ABSTRACT

Adult albino mice were exposed to 2° C in groups for varying time periods prior to challenge intraperitoneally with 1 LD_{50} of Klebsiella pneumoniae. Animals exposed to the low temperature for up to five days prior to challenge showed no significant differences in mortality from the controls. Statistically significant survival ratios were obtained if the mice were exposed to cold for more than five days prior to challenge. No deaths were observed in comparable specifically immunized groups of animals.

PUBLICATION REVIEW

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EFFECT OF ACCLIMATIZATION TO 2° C ON HOST SUSCEPTIBILITY TO KLEBSIELLA PNEUMONIAE CHALLENGE*

SECTION 1. INTRODUCTION

The effect of low ambient temperature on host resistance to disease has been investigated since the time of Pasteur, Joubert and Chamberland (1878), who demonstrated that chilling of chickens could lower their resistance to anthrax infection. Most of the reported results have been related to the effects of acute low temperature exposure and few reports are concerned with long term cold exposure on host resistance to experimental disease.

The following report is a study of the effect of acclimatization to 2° C on the ability of mice to resist an acute challenge with Klebsiella pneumoniae administered parenterally.

SECTION 2. SUMMARY

Adult albino mice were exposed to 2° C in groups for 0, 1, 2, 3, 5, 10, 16, 33 and 50 days before challenge with 1 LD_{50} of Klebsiella pneumoniae. The mortality ratios observed for the above times of cold exposure were 10/20, 10/20, 12/20, 4/20, 6/20, 2/20, 3/20, 2/20 and 4/20 respectively. The 21° C control mortality ratio was 9/20. Statistical analysis by chi square procedure indicated that cold exposure for more than five days prior to challenge resulted in statistically significant survival. No deaths were observed in comparable specifically immunized groups of mice.

SECTION 3. MATERIALS AND METHODS

Adult albino mice (Mus musculus) obtained from local sources were used. The average weight of the animals was 20 to 21 gm at the initiation of the experiment and both sexes were employed in a random fashion.

* This research was conducted in accordance with the "Principles of Laboratory Animal Care" of the National Society for Medical Research.

Klebsiella pneumoniae was obtained from departmental stock cultures. Single colonies were isolated on Difco heart infusion agar plates and inoculated into Difco tryptose phosphate broth. The bacteria were allowed to grow for 18 hours at 37° C. The number of organisms per ml was determined by reference to a standard curve relating numbers of viable organisms per ml to the turbidity of the suspension. Readings were obtained in calibrated 13 x 100 mm curvettes placed in a Klett-Summerson photoelectric colorimeter with a blue filter. The bacterial suspension was diluted with sterile 0.15 M NaCl solution to yield a suspension containing 1250 organisms per ml. The bacterial challenge in the experiments was 125 organisms per mouse and was administered intraperitoneally in 0.1 ml volume.

The vaccine was composed of formalin killed organisms (Marcus et al, 1961) and the mice were immunized by five injections given every other day. The immunizing dose was 180×10^6 organisms given intraperitoneally in a volume of 0.1 ml. The challenge dose of viable organisms was given seven days after the last immunizing dose.

Mice were exposed to $2° \pm 1.5°$ C for a varying number of days prior to challenge. The animals were caged in groups of 10 and were given water and food ad libitum. The cold exposed animals were caged with sawdust bedding barely sufficient to cover the cage bottoms.

SECTION 4. RESULTS

Preliminary experiments with small numbers of normal animals indicated a trend toward decreased susceptibility to 1 LD_{50} challenge dose (125 organisms) if the animals were cold exposed for periods greater than 10 days prior to challenge. This effect was not observed when larger challenge doses were employed, since this resulted in uniformly fatal outcome in all groups of animals. The immunized animals were protected against death during the period of the experiments.

The definitive experiment was completed and the results are shown in Table I. In general it is seen that normal animals exposed to 2° C for 10, 16, 33 or 50 days before challenge with 1 LD_{50} dose of K. pneumoniae gave mortality ratios significantly different from the animals challenged after 0, 1, 2, 3 and 5 days of exposure to 2° C. The room temperature controls showed 9/20 deaths. Since the experiment was designed to give the same challenge suspensions to all groups on the same day, only one normal control is needed.

Chi square analysis of the data comparing the mortality ratios of the various groups of cold exposed animals to the mortality ratio of the 21° C normal control groups yields probability values ranging from significant (.05) to highly significant (.001) for the animals exposed to 2° C for 10, 16, 33 or 50 days prior to challenge.

The specifically immunized groups did not have any deaths whether cold exposed or not.

There were no differences in the rates at which the deaths occurred and no deaths were observed after eight days post challenge. The experiment was terminated 14 days post challenge.

TABLE I

Fourteen Day Mortality Ratios of Mice Challenged Intraperitoneally With 1 LD_{50} of Klebsiella pneumoniae After Exposure to 2° C

Groups	Days of Exposure Prior to Challenge								
	0	1	2	3	5	10	16	33	50
2° C									
Normal animals	10/20	10/20	12/20	4/20	6/20	2/20	3/20	2/20	4/20
Specifically immunized animals	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
21° C (control)									
Normal animals	9/20								
Specifically immunized animals	0/20								
P (X^2)	(.8-.7)	(.8-.7)	(.5-.3)	(.1-.05)	(.5-.3)	(.01-.001)	(.05-.02)	(.01-.001)	(.1-.05)

SECTION 5. DISCUSSION

When Klebsiella pneumoniae is employed as the challenge agent in experimental infections, it is possible to obtain quite accurately predictable mortality ratios. The LD_{50} of the organism is approximately 75 to 100 viable bacteria per mouse (Marcus et al, 1955; 1960). If one employs excessive challenge doses, a uniformly fatal infection occurs regardless of the ambient temperature at which the animals are kept.

In general one would expect that exposure of an animal to low ambient temperatures would result in decreased host resistance. If the exposure to cold occurred on an acute basis and significant decrease in host resistance to challenge was observed, the next logical procedure would be to repeat the experiment in animals that were acclimatized to the low temperature prior to challenge.

In 1942, Mills and Schmidt reported that mice kept at 20° C for three weeks or more before challenge with pneumococci survived twice as long as mice kept at 32° C. In contrast, Junge and Rosenthal (1948) observed that mice kept for several days at 18° C before challenge with pneumococci had 92 per cent mortality compared to 53 per cent mortality among mice kept at 31° C. Recently Previte and Berry (1962) reported that two weeks of acclimatization to 5° C did not alter the survival of mice challenged with avirulent salmonellae.

The present investigation shows that acclimatization to 2° C for periods longer than five days prior to challenge with 1 LD_{50} dose of K. pneumoniae results in enhanced host resistance. As stated earlier, this effect is not seen when large challenge doses are employed. Previous results from our group (Marcus et al, 1961) indicated that acclimatization to 2° C would enable singly caged specifically immunized mice to survive K. pneumoniae challenge in a manner similar to animals kept in groups. The same experimental results showed that normal animals had uniformly high death rates regardless of acclimatization or acute exposure to 2° C and also regardless of whether they were caged singly or in groups.

The conflicting results that have been reported concerning the benefits or detriment of long term cold exposure can be due to the challenge agents employed, the doses given, the routes of challenge, the degree of cold exposure and the time allowed for acclimatization. With regard to the last

factor, no one knows when an animal is acclimatized. Is 5 days sufficient or is a 14-day period necessary? Or, does acclimatization require even longer periods of exposure? The animal species is a factor in consideration of answers to the above acclimatization periods.

Schwabe et al (1938) showed that the metabolism rate of rats increased 15 to 16 per cent between the 15th and 30th day of exposure to 8° C and this rate maintained itself without change for as long as 60 days post exposure to the cold.

Lee (1942) reported that rabbits had to be habituated to a given temperature for three weeks if the basal metabolic rate was to be used as a measure of an effect of a superimposed condition.

Denison and Zarrow (1954), working with rats, presented results that suggested there is an increased amount of corticosteroid present during the initial phase of cold exposure. They found that such exposure resulted in a marked decrease in circulating eosinophils in the first 6 hours post exposure, but that normal eosinophil counts were obtained by 48 hours post exposure. In general the levels of circulating eosinophils remained high for the duration of 90 days exposure to 2° C.

Results of our own group (Marcus et al, 1963) indicate that the adrenal gland of mice hypertrophies, but does not show evidence of increased activity as measured by thymus gland involution during 45 days of exposure to 2° C. Eosinophil counts will be carried out to further explore this point.

It may be inferred from the above information that (a) a parameter of acclimatization to cold for a given animal species may not apply to other species, (b) the period of acclimatization to cold varies from species to species, and (c) the acclimatization period for a given species should be defined for proper interpretation of data.

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