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EFFECT OF ACUTE AND CHRONIC EXPOSURE TO LOW TEMPERATURES ON SURVIVAL OF MICE CHALLENGED WITH KLEBSIELLA PNEUMONIAE

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ABSTRACT

Normal and immunized mice were subjected to acute and chronic stress of 2° C ambient temperature. Singly caged mice were adversely affected by the acute cold stress, but mice chronically cold-stressed were significantly protected by the immunization whether caged in groups or singly. Chronic cold stress did not decrease the ability of the animals to form agglutinin antibody.

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Although numerous investigators (Thompson, 1938; Mills and Schmidt, 1942; Moragues and Pinkerton, 1944; Cottingham and Mills, 1943; Muschenheim, et al., 1943) have reported results concerning the effect of temperature on resistance mechanisms, little information is available relating to the effects of acute and chronic cold stress on host resistance mechanisms. In addition, workers have found that cold environments favor survival when animals have been challenged with Pneumococcus type I (Mills and Schmidt, 1942), tetanus toxin (Ipsen, 1952), or influenza virus (Sulkin, 1945). It should be mentioned that these latter investigators employed ambient temperatures of 20°, 6° and 16° C, respectively. The following experiments were conducted to determine the effect of acute and chronic exposure to 2° C ambient temperatures on the host's ability to withstand experimental infection with Klebsiella pneumoniae.

MATERIALS AND METHODS

Adult albino mice (Mus musculus) obtained from local sources were used in the experiments. Both sexes were used in a random fashion.

Klebsiella pneumoniae obtained from departmental stock cultures was maintained on heart infusion blood agar slants (Difco). A single colony obtained by isolation procedures was inoculated into tryptose phosphate broth (Difco) and incubated at 37° C for 18 hours. The number of organisms per ml was determined by reference to a standard curve relating the numbers of organisms per ml to the turbidity of the culture. A Klett-Summerson photoelectric colorimeter with a blue filter was employed, and the readings were obtained in 13 x 100 mm calibrated cuvettes. The broth culture was diluted with a sterile 0.15 M NaCl solution to yield the desired inoculum size. The bacterial challenge in all experiments was administered intraperitoneally in 0.1 ml volumes.

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The ambient temperatures chosen for these experiments were 21° and 2° C. The temperatures of these rooms did not vary more than ± 1.5° C during the time of the experiment as determined by continuous temperature monitoring with a calibrated temperature recording instrument (Tempscribe, Bacharach Industrial Instrument Co., Pittsburgh, Pa.).

Vaccines were prepared from broth cultures of K. pneumoniae. A stock culture, kept in the refrigerator, was streaked to blood agar plates. Smooth mucoid colonies were picked after 18 hours incubation at 37° C. Formalin was added to a final concentration of 0.5%. The organisms remained in contact with the formalin for 18 hours at 37° C and were then washed three times with saline by centrifugation procedures. The residue was resuspended in saline and quantitated by employing a counting chamber. Viability tests (culture on agar and in broth) were uniformly negative.

Mice were immunized by a series of five injections given every other day. The immunizing dose was 180×10^6 dead organisms given intraperitoneally in a 0.1 ml volume. One week was allowed to lapse before challenge with viable organisms.

Agglutinin titers. Seven days after the last immunizing injection, mice were sacrificed by decapitation and the blood was collected in one pool. The blood was allowed to clot two hours at room temperature and then was placed overnight in the refrigerator. The serum was recovered by centrifugation. Serial twofold dilutions of the serum were made in saline (0.5 ml). A constant volume (0.1 ml) of dead organisms was added. The tubes were incubated at 37° C in a water bath for two hours and then were placed overnight in the refrigerator. The degree of agglutination was observed with the aid of a mirror. The last tube showing agglutination was recorded as the end point.

RESULTS

Acute exposure experiments. Mice were immunized at room temperature and then were challenged with varying numbers of K. pneumoniae. Immediately following this procedure the mice were transferred to an ambient

temperature of 2° C. The control mice groups were kept at 21° C. The results are summarized in Table I. It is seen that immunization was effective in the animals that were grouped. In contrast, the immunization procedure was only slightly effective in mice singly caged. The nonchallenged stress controls placed at 2° C did not die when grouped but did when singly caged, suggesting that "huddling" of animals enables more favorable outcome when animals are acutely stressed by low ambient temperatures.

Chronic exposure experiments. Mice were placed at 2° or 21° C for periods of 45 days before being immunized. One week after the last immunizing injection, the mice were challenged with varying numbers of viable K. pneumoniae. The results are summarized in Table II. It is seen that mice are significantly protected by the immunization procedure. The titer of agglutinin antibody formed by mice chronically exposed to 2° C is comparable to that formed by animals kept at 21° C. It is interesting to note that no significant differences in mortality of normal or immunized mice are apparent whether the mice are grouped or singly caged. It can be concluded from these results that mice chronically exposed to an ambient temperature of 2° C are able to form agglutinin antibody and that the immunization procedure offers significant protection against the challenge organisms. In contrast, normal mice chronically exposed to 2° C are adversely affected by K. pneumoniae (i. e., smaller numbers of organisms can cause increased mortality whether the animals are grouped or caged individually).

DISCUSSION

It is apparent from these experiments that mice chronically exposed to low ambient temperatures respond favorably to immunization procedures. The immunization procedure enables more mice to survive a bacterial challenge whether the animals are caged in groups or singly. In contrast, the acute exposure experiments favor the grouped mice over the singly caged mice with respect to survival from bacterial challenge and/or extreme cold stress. A logical explanation is that grouped mice "huddle" in an effort to conserve body heat loss to the environments, and thus they reduce the cold stress (Marcus, et al., 1961).

The change in animal weights during the time of chronic exposure to 2° C

TABLE I

EFFECT OF ACUTE COLD STRESS (2° C) ON MORTALITY OF MICE CHALLENGED WITH KLEBSIELLA PNEUMONIAE. UNADAPTED ANIMALS INJECTED AT ROOM TEMPERATURE, THEN PLACED AT NOTED TEMPERATURES.

	Ambient Temperatures					
Number of Organisms	21°C (grouped)		2° C (grouped)		2° C (single)	
	normal	immunized	normal	immunized	normal	immunized
4.3	8/10*	0/10	7/8	0/10	8/8	6/10
43	9/10	0/10	9/10	0/10	9/10	7/10
430	10/10	0/10	10/10	0/8	10/10	6/10
4300	10/10	0/10	10/10	1/8	10/10	6/10
Agglutinin titer **	0	1/32	0	1/32	0	1/32

* 7 day mortality (dead/total).
** Titer before challenge.

TABLE II

EFFECT OF CHRONIC COLD STRESS (2° C) ON MORTALITY OF MICE CHALLENGED WITH KLEBSIELLA PNEUMONIAE. ANIMALS MAINTAINED FOR 45 DAYS, AND IMMUNIZED, AT NOTED TEMPERATURES.

	Ambient Temperatures					
Number of Organisms	21° C (grouped)		2° C (grouped)		2° C (single)	
	normal	immunized	normal	immunized	normal	immunized
5.6	3/10*	0/10	10/10	0/10	7/9	0/10
56	4/10	0/10	9/9	1/10	7/8	0/10
560	10/10	0/10	10/10	2/10	6/6	1/10
5600	10/10	0/10	10/10	2/10	9/9	1/10
LD_{50} (95% Confidence limits)	56 (52-59)	-	-	51×10^4 (49×10^4 - 52×10^4)	4.1 (3.8-4.4)	-
Agglutinin titer**	0	1/32	0	1/16	0	1/16

* 7 day mortality (Dead/total).
**Titer before challenge.

was followed. In Figure 1 it can be seen that these animals showed a significant increase in average animal weight during the 45 days of exposure. In contrast, the body weight increase of animals kept at 21° C for the same periods showed a slow constant increase. The 2° C mice showed the greatest weight increase between 10 and 20 days after the beginning of exposure. The weight curve then appeared to level off to a weight increase rate identical to that of the 21° C mice but at a higher absolute level. The cold-exposed mice grew thicker coats of body hair, and their skin appeared to be flabby compared to the 21° C mice. Cross-sections taken through the nuchal skin region showed no overt excess deposition of fat.

Antibody formation was not inhibited by animals chronically exposed to low ambient temperatures. Essentially the same agglutinin antibody level was attained both by animals immunized in the cold and those immunized at 21° C. Ipsen (1952) reported that antibody production is inhibited by cold if small immunizing doses are employed; this effect is not observed if large doses of antigen are administered. In our experiments varying doses of antigen were not employed; however, the immunizing dose used was not considered "large" since it is the standard dose employed routinely in our laboratory.

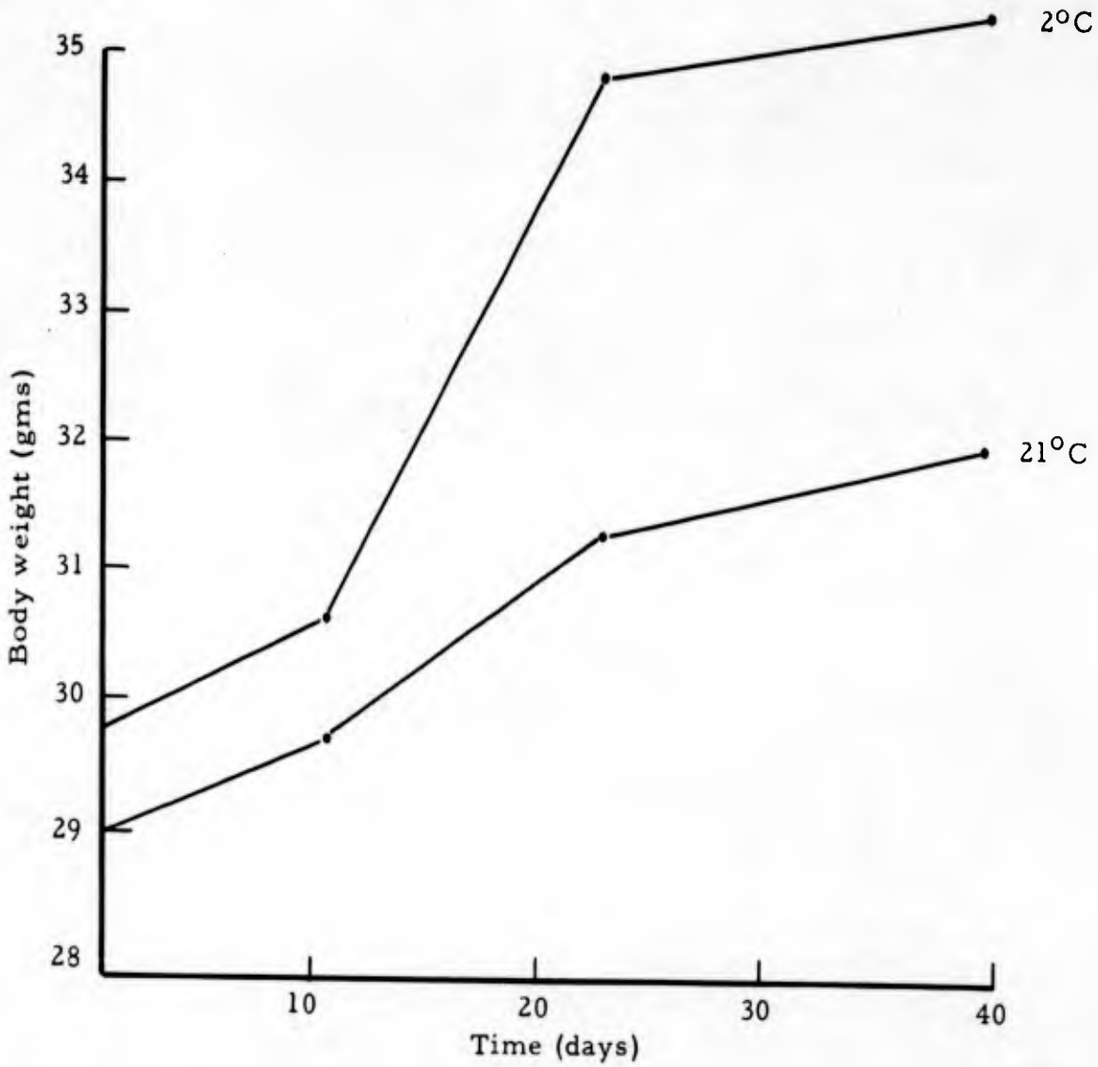

Figure 1. Effect of chronic low temperature (2°C) stress on average body weights of mice.

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