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EFFECT OF AMBIENT TEMPERATURE AND CHLORPROMAZINE TREATMENT ON RESISTANCE OF MICE CHALLENGED WITH KLEBSIELLA PNEUMONIAE

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ABSTRACT

The hypothesis that increased metabolic rates enhance the host resistance to infection was investigated. Chlorpromazine was used to induce the state of hypothermic stress without compensatory increase in metabolic rates. The results of the experiments showed that the ambient environment influences the toxicity of the drug itself. Chlorpromazine enhanced the death of mice challenged with Klebsiella pneumoniae, and an interpretation could be that the lowering of metabolic rates as a result of the drug injection was contributory to this effect.

EFFECT OF AMBIENT TEMPERATURE AND CHLORPROMAZINE TREATMENT ON RESISTANCE OF MICE CHALLENGED WITH KLEBSIELLA PNEUMONIAE*

Maintenance of a thermal steady state by animals is accomplished by several compensatory mechanisms. Poikilothermic animals achieve the thermal steady state by allowing excess heat to escape from or to enter their bodies depending on environmental temperatures. It is apparent that under these conditions only the metabolism and rates of chemical reactions change as ambient temperatures change. This type of compensatory mechanism is not physiologically possible for homeothermic animals. Homeotherms achieve the thermal steady state by compensatory mechanisms which include heat production, insulation and evaporation. It is apparent that homeotherms, under conditions of low environmental temperatures, have limited use for evaporative mechanisms for maintenance of the thermal steady state. When a homeotherm is subjected to low ambient temperatures, the maintenance of the thermal steady state appears to be the result of insulation and increased heat production. Only under conditions of chronic low temperature exposure are insulative mechanisms of paramount importance, while increased heat production mechanisms function at all stages of exposure.

It has been shown by Herrington (1940) that small laboratory animals (mice, rats) have only a narrow range of environmental temperature over which constancy of the metabolic rate is maintained. Above and below this temperature range the metabolic rate increases. The increase in metabolic rate at temperatures below this range of "environmental neutrality" is a result of compensation for the increased heat loss (Burton and Edholm, 1955).

The hypothesis upon which the experiments to be reported were based is that increased metabolic rates resulting from exposure to ambient temperatures lower than that of "environmental neutrality" increases the host resistance to challenge by infectious agents. Chlorpromazine was used to induce hypothermic stress without compensatory increases in metabolic rates.

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MATERIALS AND METHODS

Klebsiella pneumoniae obtained from departmental stock cultures was maintained on heart infusion blood agar slants (Difco). Single colonies obtained by isolation procedures were 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 a suspension containing 680 organisms per ml. The bacterial challenge in all experiments was 68 organisms per mouse and was administered intraperitoneally in 0.1 ml volumes.

Chlorpromazine (CPZ) (Smith-Kline-French) was dissolved in 0.15 M NaCl solution to a final concentration of 200 μg per ml. Animals were injected (40 μg/0.2 ml) either subcutaneously (nuchal area) or intraperitoneally.

Adult albino mice (Mus musculus) obtained from local sources were used in the experiments. The average weight of the animals was 21 g; both sexes were used in a random fashion.

After the various treatments and challenge procedures the mice were placed at different ambient temperatures. The average temperatures of the various animal rooms were 2°, 21° and 37° C. The temperatures of these rooms did not vary more than ± 1° C during the time of the experiments as determined by continuous temperature monitoring with a calibrated temperature recording instrument (Tempscribe, Bacharach Industrial Instrument Co., Pittsburgh, Pennsylvania).

Mouse rectal temperatures were obtained by insertion of a probe 2 cm into the rectum; these values were recorded with an Electric Universal Thermometer Type TE3 (Probe Model R.M. 4, Chemical and Pharmaceutical Industry Co., Inc., New York City, N. Y.)

RESULTS

In order to evaluate the effect of CPZ in experiments of this nature, it was necessary to determine if the drug was bacteriostatic toward K. pneumoniae. The results indicated that the drug was nonhemolytic in concentrations up to 25 μg/ml and nonbacteriostatic in concentrations up to 250 μg/ml. The latter figure was considerably higher than the calculated values for the final CPZ concentration per ml of blood in the mice when the animals were given 40 μg. This dosage of 40 μg per mouse was sufficient to induce hypothermia as determined by a fall in rectal temperature.

Several experiments were conducted to test the hypothesis that increased metabolic rates and heat production by mice exposed to low ambient temperatures would increase the host's resistance to challenge by an infectious agent. The results of a typical experiment are presented in Figures 1 through 5. Since experiments were conducted simultaneously at the various ambient temperatures, the same K. pneumoniae control mortality curves are included in each figure for the respective temperatures.

When mice were treated with a single subcutaneous injection of CPZ followed immediately by the intraperitoneal challenge of K. pneumoniae and placed at the indicated temperatures, the mortality curves shown in Figure 1 were obtained. It is seen that at 2° and 21° C, treatment with CPZ alone did not cause any significant numbers of deaths; however, at 37° C this same treatment resulted in mortality curves almost identical with those obtained with mice challenged with K. pneumoniae alone or challenged with the organism and treated with a single injection of CPZ. Also, at 2° and 21° C the mice receiving both K. pneumoniae and CPZ exhibited mortality curves similar to the K. pneumoniae-challenged control mice.

Figure 2 shows mortality curves of mice treated subcutaneously with CPZ and then challenged with K. pneumoniae. In this case, treatment with the drug was repeated every six hours. It is seen that CPZ treatment resulted in enhanced mortality of the challenged mice kept at 2°, 21° or 37° C when compared to the K. pneumoniae controls. Furthermore, mice treated repeatedly with CPZ alone exhibited greater mortalities when kept at 2° or 37° C compared to the mice receiving only CPZ and kept at 21° C. The enhanced mortality of the CPZ treatment alone was most prominent in mice kept at 2° C. It may be concluded that repeated CPZ treatment given to

K. pneumoniae challenged mice increased mortality when these mice are kept at 21° C; however, the increased mortality of mice treated, challenged and kept at 2° or 37° C can be the result of the CPZ treatment interfering with other mechanisms not related to bacterial challenge, since deaths occur in the absence of K. pneumoniae challenge.

If the mice received CPZ at the time of bacterial challenge and every 24 hours after challenge with the organisms, mortality curves resembling single CPZ treatment and K. pneumoniae challenge are obtained (compare Figure 3 with Figure 1). A likely interpretation is that CPZ effects have worn off and the conditions resemble that obtained when only a single injection is given. Evidence that the hypothermic effects of CPZ are no longer present at 24 hours is shown in Figure 6. It is seen here that treatment of mice with the drug results in a hypothermia from which the animals recover within 24 hours. This is in agreement with the work of Courvoisier, et al. (1953).

When mice are given a single injection of CPZ intraperitoneally followed immediately by K. pneumoniae challenge, mortality curves shown in Figure 4 are obtained. It is seen that drug treatment via this route appears to enhance the mortality of mice as compared to CPZ given subcutaneously under the same conditions. Although mice treated with CPZ alone intraperitoneally and kept at 21° C do not die, the intraperitoneal treatment results in greater mortality of mice kept at 2° C as compared to mice treated subcutaneously and kept at 2° C (compare Figure 4, 2° C, with Figure 1, 2° C). It can be concluded that the mortality increase of K. pneumoniae-challenged and CPZ treated mice is dependent on the route of administration of CPZ in addition to the ambient temperature.

In order to investigate all aspects of chlorpromazine treatment, an ancillary experiment included mice pretreated with CPZ given subcutaneously every 24 hours for 7 days prior to challenge with K. pneumoniae. Mortality curves are shown in Figure 5. The mice were pretreated at 21° C, challenged with the organisms and then placed at the specified ambient temperatures. It is seen that pretreatment did not significantly affect the host susceptibility to K. pneumoniae at 2° and 21° C since treated mice exhibited mortality curves similar to the untreated mice; however, the pretreatment appeared to enhance the mortalities of mice kept at 37° C.

●—● K. pneumoniae control; o—o CPZ Control; Δ·····Δ CPZ + K. pneumoniae

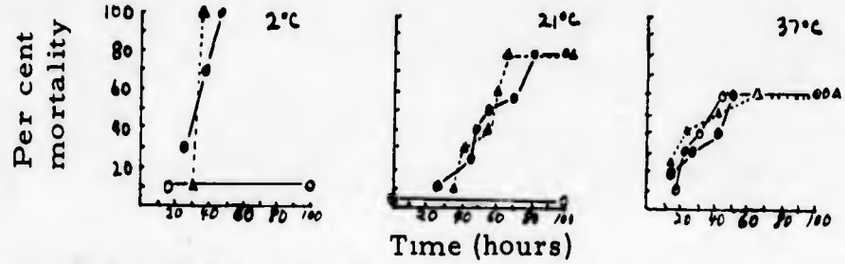

Figure 1. Effect of a single chlorpromazine injection (subcutaneous) on the mortality of mice challenged with K. pneumoniae. (Legend above applies to Figures 1 to 5)

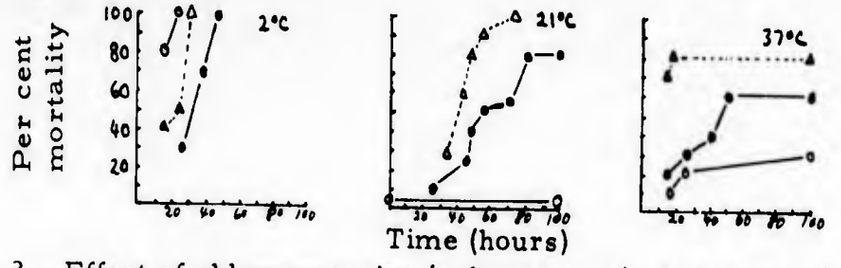

Figure 2. Effect of chlorpromazine (subcutaneous) given every 6 hours on the mortality of mice challenged with K. pneumoniae.

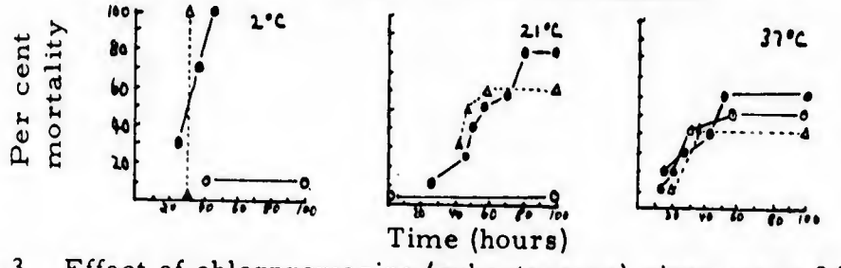

Figure 3. Effect of chlorpromazine (subcutaneous) given every 24 hours on the mortality of mice challenged with K. pneumoniae.

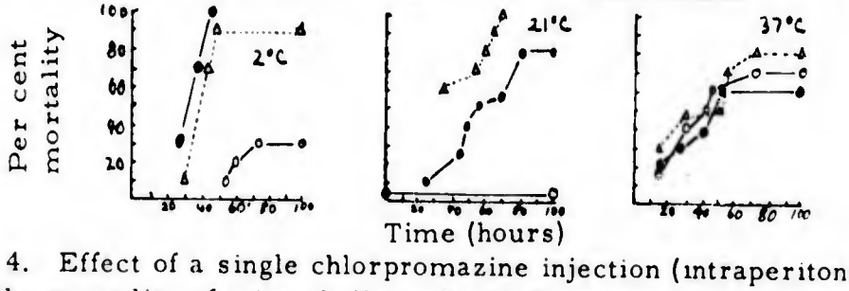

Figure 4. Effect of a single chlorpromazine injection (intraperitoneal) on the mortality of mice challenged with K. pneumoniae.

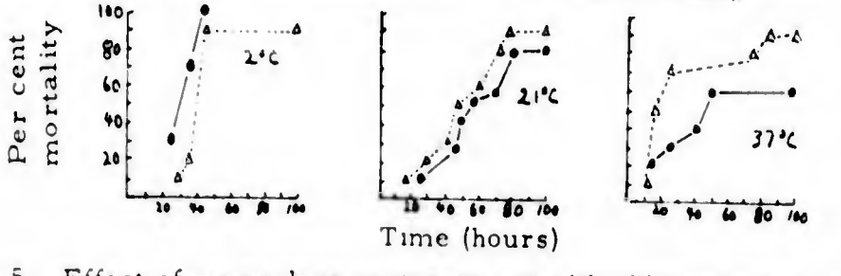

Figure 5. Effect of seven days pretreatment with chlorpromazine (subcutaneous) on the mortality of mice challenged with K. pneumoniae.

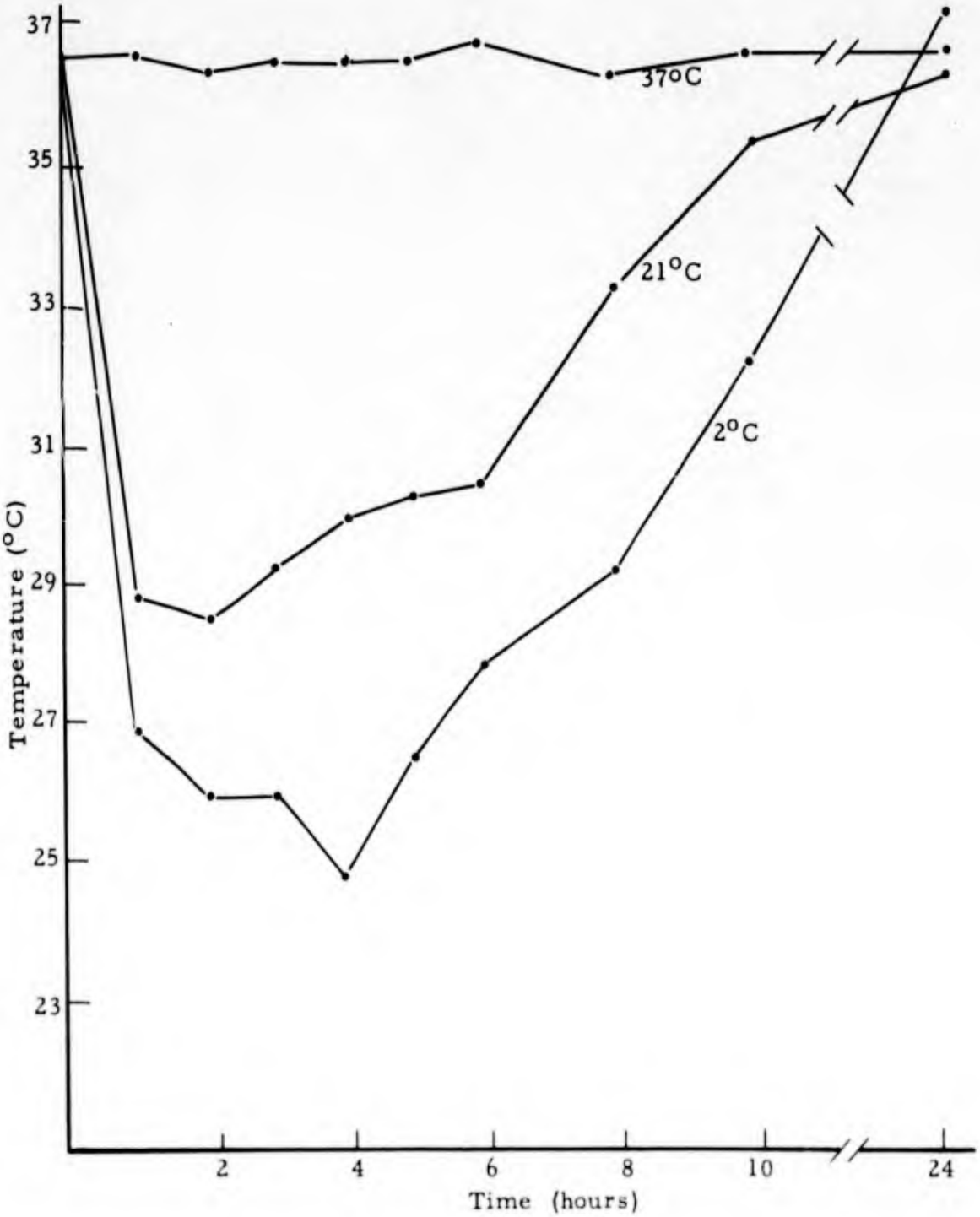

Figure 6. Effect of a single injection subcutaneously of chlorpromazine (40 μg) on the rectal temperatures of mice kept at various ambient temperatures.

DISCUSSION

A general observation of these experiments was that mice treated with CPZ (but not challenged with K. pneumoniae) and kept at 37° C died at rates as fast or faster than mice challenged with the organism alone or mice treated with the drug and challenged with the organism. However, in the absence of bacterial challenge the toxicity of CPZ toward mice kept at 2° C was dependent on the route of administration and frequency of treatment. For example, CPZ toxicity resulted in final mortalities of 30% in mice receiving a single injection intraperitoneally, in contrast to final mortalities of 10% in mice receiving a single injection subcutaneously. In addition, mice receiving repeated injections at six-hour intervals showed mortalities of 80% at 12 hours and 100% at 18 hours in contrast to final mortalities of 10% in mice receiving the drug every 24 hours. Similar results showing increased toxicity of CPZ at low and high temperatures have been reported by Dandiya, et al. (1960); however, it is noted that these authors employed doses of CPZ considerably higher than those used in the present investigation.

The use of chlorpromazine as an agent to induce hypothermic stress without compensatory increases in metabolic rate may be based on false premises (i.e., that this effect is the major property of the drug). The properties and action of the drug have been investigated by Courvoisier, et al. (1953), who have described numerous effects of the drug. Since the publication of this work, much material has appeared concerning the varied effects of the drug. However, the dose of drug employed in our experiments was sufficient to induce a significant hypothermia without overt signs of other effects reported (e.g., central nervous system depression, increased heart and respiration rates, loss of responsiveness to noxious stimuli, loss of normal ingestive and behavioral motions). The animals remained grossly normal in all these respects. It was found in pilot experiments that doses of CPZ from 22 to 2000 μg per mouse caused degrees of alteration in behavior (physically and physiologically) that were directly proportional to the dose employed. Therefore it was felt that the dose of 40 μg per mouse used in these experiments produced the desired hypothermia without notable increase or change in metabolic rates and with a minimum of side effects.

With the use of CPZ to induce the control situation for the hypothesis tested by these experiments, it was shown that treatment with CPZ appeared

to enhance the mortality of mice challenged with K. pneumoniae. Conversely, one might interpret the results to mean that alterations in metabolic rates influence the host's susceptibility to disease. Since deaths of mice occurred with CPZ treatment alone at temperatures of 2° or 37° C but not at 21° C, and since the dosage of CPZ employed in these experiments exerted hypothermic effects up to six hours, it follows that one should limit speculations to the ambient temperature of 21° C and to the treatment schedule of an injection of CPZ every six hours (Fig. 2) for proper evaluation of the hypothesis. However, to fully investigate the hypothesis, one would need to have drugs or procedures available to maintain the metabolic rate of the animals constant without core temperature changes when the animals are subjected to progressively lower ambient temperatures. This drug or procedure would of necessity have to be specific in its action and without undue side effects.

It is of interest to note that other workers (Grosz and Norton, 1959; Greenberg and Ingalls, 1960) have reported that injection of CPZ enhances the lethality of Salmonella enteritidis infection in mice but that the susceptibility of mice to Salmonella typhimurium was not significantly altered (Greenberg and Ingalls, 1960). Other workers (Ludany, et al., 1956; Meier, et al., 1957) have reported that CPZ may act as depressors of phagocytic activity. This aspect of the effects of the drug was not investigated in the present experiments and may or may not have contributed to the enhanced susceptibility of the mice to K. pneumoniae. Perhaps the decreased phagocytic activity could be due to alterations in metabolic rates induced by CPZ.

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