Technical Papers

The Effect of Environmental Temperature on Cortisone Toxicity for Mice[1]

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In the course of a study now being conducted on the effect of cortisone and other compounds on mice systemically infected with *Candida albicans,* it became apparent that the mortality rates of control animals varied with the season of the year. It was therefore felt pertinent to ascertain what effect different environmental temperatures had on infected mice and on uninfected, treated controls; this report concerns itself with the latter.

Groups of 10 Swiss mice (departmental stock), 18–22 g, were placed at incubation temperatures of 35° to 37° C, 25° to 29° C, and −5° to 7° C. Half of these animals were inoculated intramuscularly with 0.5 mg (0.1 ml) of cortisone acetate (Merck)[2] every 48 hr

TABLE 1

SURVIVAL OF MICE INOCULATED WITH 0.5 MG OF CORTISONE ACETATE EVERY 48 HR AND INCUBATED AT VARIOUS TEMPERATURES

Temp (°C)	Sex	No. surviving								
		1	2	3	4	5	6	7	8	Days
35°–37°	M	10	10	10	8	7	7	5	3	
25°–29°	M	10	10	10	9	6	6	6	6	
−5°– 7°	M	10	10	10	10	10	10	10	10	
35°–37°	F	10	10	10	9	9	9	8	7	
25°–29°	F	10	10	10	10	9	9	9	9	
−5°– 7°	F	10	10	10	10	10	10	10	10	
Controls, untreated										
35°–37°	M	10	10	10	10	10	10	10	10	
25°–29°	M	10	10	10	10	10	10	10	10	
−5°– 7°	M	10	10	0	0	0	0	0	0	
35°–37°	F	10	10	10	10	10	10	10	10	
25°–29°	F	10	10	10	10	10	10	10	10	
−5°– 7°	F	10	10	0	0	0	0	0	0	

for 8 days, initiating this treatment on the first day. Untreated animals were maintained as controls. Table 1 records the numbers of survivors in all groups of mice studied.

From these data one may infer the following:

1. The toxic effects of cortisone were enhanced by an increased environmental temperature (35° to 37° C).

2. This enhanced toxicity was more manifest in male mice. This was evident at the 35° to 37° C and 25° to 29° C temperature ranges. This effect was also reported by Ingle (*1*) for male and female rats inoculated with "cortin."

3. A cold temperature (−5° to 7° C) eliminated the toxic effects of the cortisone manifest at higher environmental temperatures.

4. This dosage of cortisone eliminated the deleterious effects of the cold. Thus, all treated mice, male and female, survived the 8-day incubation period, whereas all the controls were dead by the second day.

This work was repeated with essentially the same results.

These experiments lack the refinement of humidity control and maintenance of a constant oxygen tension in the incubators, which may be significant in modifying the mortality rates of the mice. The work should be repeated by those having access to temperature- and humidity-controlled cabinets that are also air-circulated.

Numerous workers who have been studying the effects of cortisone on infected animals determine the toxic levels for cortisone for a certain schedule and use this standard for all future experiments without repeating this control portion of the work. It is our contention that controls of uninfected, treated groups of animals are required for *each* experiment. The alternative is to perform such experiments under conditions where temperature, humidity, and air circulation can be controlled.

It is also conceivable that these temperature effects might be significant in the clinical use of cortisone. It is not known to this author that such applications have been made.

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[2] The generosity of Merck & Co. in supplying cortisone acetate is gratefully acknowledged.

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Adsorption of Serum Lipids by Montmorillonite

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The fact that soils high in clay retain much greater amounts of organic matter than those low in clay has led to several investigations dealing with the adsorption capacity of clays. Among them montmorillonite is very effective because of its high ion exchange and swelling capacities. Thus montmorillonite adsorbs large protein molecules, such as albumin, gelatin, hemoglobin

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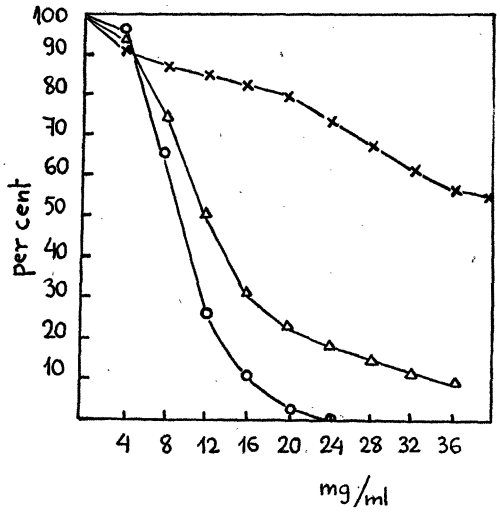

FIG. 1. The effect of montmorillonite on serum protein (×—×), total cholesterol (○—○), and phospholipids (△—△). Mean values from 7 sera. Abscissa: mg bentonite/ml serum. Ordinate: percentage of control sample.

(*1, 2*), and humic colloids of soil (*3*). The adsorption mechanism has been discussed by several authors (*1–5*). Because the adsorption of organic colloids in montmorillonite is most effective at low pH, it is possible that a reaction takes place between the basic groups of the proteins and the negative charges on the clay (*2, 6*). Montmorillonite combined with proteins has a much smaller base exchange capacity than uncombined montmorillonite, which shows that a blockage of exchangeable groups must occur. The properties of the proteins combined with this clay mineral are also altered. This is shown by the high resistance of the protein-montmorillonite complex to hydrolysis by proteolytic enzymes (*7*), and to decomposition by soil microorganisms (*8*), as well as by the lowering of the isoelectric point of the protein after the addition of bentonite (*9*).

The adsorption capacity of montmorillonite has been used by Hansen (*10*) for the removal of antiproteolytic serum factors, especially lipoproteins—e.g., diphtheria antitoxin purification. According to him montmorillonite added to diluted serum removes cholesterol and lipids, besides remnants of fibrin and euglobulin, without any appreciable change in the antitoxin content of the serum. Serum lipids also seem to be responsible for the nonspecific antistreptolysin-O reactions (*11*), and for this reason an attempt to remove these serum lipids with montmorillonite was made (*12*). These experiments have confirmed the statements made by Hansen, and the data presented here show the effect of montmorillonite on the content of cholesterol, phospholipids, and proteins of normal human serum.

Montmorillonite suspensions of varying concentrations were made in distilled water and added to normal human serum diluted with equal parts of physiological saline. The mixture was shaken gently and allowed to stand for half an hour at room temperature and then centrifuged at 2500 rpm. The clear supernatant fluid was then analyzed for total cholesterol, phospholipids, and proteins. The results so obtained are presented in Fig. 1. The diagram shows that, when used in small concentrations and at a slightly alkaline pH (about 8.0), montmorillonite adsorbs all the cholesterol, 80–85% of the phospholipids, and 15–20% of the proteins. Electrophoretic analyses of the bentonite-treated sera showed that the greatest changes occurred in the β-globulin fraction of the serum, for this was reduced to about 20%, when 20 mg of montmorillonite/ml of serum were used.

According to Blix *et al.* (*13, 14*), among others, the serum lipids are supposed to occur mainly in the β-globulin fraction of the serum. When calculating the amount of lipids and proteins adsorbed in this experiment, it thus seems likely that the component(s) which are adsorbed represent a lipoprotein containing about 25% lipid material, and accordingly the part (15–20%) of the phospholipids that is not adsorbed by montmorillonite is not combined as lipoprotein. It is difficult to say, however, to what extent the protein moiety of this montmorillonite-adsorbable lipoprotein complex functions as a lipid carrier also in untreated serum. This question has been to some extent elucidated by the recent investigations of Turner *et al.* (*15*). According to their ultracentrifugal studies on native serum, 70% of the serum lipids and about 20% of the proteins were found in the upper half of the ultracentrifugate column. In this connection it may also be worth mentioning that extraction of the serum lipids with acetone and ether in the cold does not remove all the phospholipids (*14*). Thus, independent of the separation method used, a fraction of the serum lipids containing, for example, the total cholesterol, can apparently be easily separated, whereas another fraction, containing a part of the phospholipids, seems to be very difficult to separate from the serum.

On the basis of the findings presented here, it is our opinion that montmorillonite could be used for the removal of lipid-containing material from different sources, as well as for investigations concerned with the interactions between lipids and proteins.

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