Toxicity of Colchicine, Di-isopropyl Fluorophosphate, Intocostrin, and Potassium Cyanide in Mice at 4°C. (18547)

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Toxicity studies at low ambient temperatures have been carried out predominantly in poikilotherms where the body temperature closely parallels that of the environment. These findings have been summarized by Fuhrman(1). Experiments conducted on mammals have been largely limited to drugs which impair thermoregulation. Thus it has been found that certain anesthetics are more toxic at low temperatures(2) while a calorific agent such as dinitrophenol is less toxic when the environmental temperature is lowered(1). In the present study, the effect of low ambient temperature on the toxicity of drugs which do not specifically impair thermoregulation was determined. The anticholinesterase, di-isopropyl fluorophosphate (DFP), and colchicine, a mitotic inhibitor, were employed for this purpose. Since the oxygen consumption of animals is significantly elevated at low ambient temperatures, it was thought to be of interest to investigate the toxicity of two anoxia-producing agents, potassium cyanide (KCN) and Intocostrin (Squibb) under these experimental conditions. Finally, the effect of acclimatization to the cold on the lethality of DFP was determined in one group of animals.

Method. All the mice used in the experiment were females ranging in weight from 18 to 32 g. Toxicity studies were carried out at 4°C ± 1.5°C and room temperature (23-25°C). The resting metabolism of the mice placed in the cold room was approximately 3 times basal(3), and these animals could not survive 24 hours without food. After 1 day at 4°C, the rectal temperature of the mice was depressed approximately 5°C, while in the succeeding 24 to 48 hours, the body temperature rose to within 2°C of normal. The concomitant weight loss for this period averaged only 3% (range was 0 to 10%), so that the quantity of the substances injected (on a basis of mg/kg) was essentially the same in both experimental and control animals. The natural mortality of 140 untreated controls was 22% for the first 24 hours following exposure to the cold, and thereafter it averaged less than 5% per day. This was compensated for in the statistical evaluation of the LD_{50}s. The mice were kept in toxicity cages supplied with food (Purina Fox Chow) and water. Each section of the cage was 7″ x 4″ x 3″ and contained 2 animals. Ten animals were injected subcutaneously at each dose and 5 dosages were employed in each determination. Colchicine, KCN, and Intocostrin were administered in saline in a volume of 0.2 ml/20 g while DFP was injected in propylene glycol in a volume of .05 ml/20 g. Control injections of 0.5 ml of saline or 0.1 ml of propylene glycol had no influence on the natural mortality of the animals. The mice were injected 24 hours after they were placed in the cold room and were maintained at 4°C until the fatalities were counted. This was done 72 hours after colchicine was administered, 48 hours after DFP, and 2 hours after KCN and Intocostrin were injected. Controls at room temperature were simultaneously injected with the same solutions. Acclimatized mice were treated with DFP 28 days after they were placed in the cold room. These latter mice were kept in ordinary cages with 25 animals per cage for 3 weeks (reducing their natural mortality for this period to 1% per day) and were placed in individual cages as described above 1 week before injection. The LD_{50}s were calculated by the method of Bliss(4) with ap-

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TABLE I. Toxicity of Various Compounds Injected Subcutaneously in Mice.

Compound	23-25°C LD_{50} (mg/kg) and std. error	4°C LD_{50} (mg/kg) and std. error	Approx. incr. in toxicity at 4°C, %
a. Colchicine	3.10 ± .20	2.32 ± .20	33
b. DFP	4.67 ± .28	3.22 ± .31	45
c. Intocostrin	.67 ± .05	.38 ± .03	76
d. KCN	6.02 ± .33	2.86 ± .16	110
e. DFP in acclimatized mice	4.43 ± .36	4.76 ± .50	None

propriate compensation made for the natural mortality of the mice in the post-injection period. The transformed dosage-mortality curves were found to be statistically parallel, so that the toxic mode of action of the drugs was assumed to be similar in both groups. Therefore the dosages calculated for the LD_{50}s in the two sets of experiments are directly comparable.

Results. The results of all experiments are shown in Table I. All drugs were significantly more toxic ($P<.01$) in mice that had been injected 24 hours after exposure to the cold than in controls (Table I-a, b, c, and d). In order to obtain the LD_{50}s at 4°C, as compared to room temperature, the dosages had to be decreased 25 to 52% depending upon the drug employed. Thus the toxicity of KCN was more than doubled and that of Intocostrin was increased 76%. The potency of the two drugs which did not interfere with oxygen utilization, colchicine and DFP, were increased 33 and 45% respectively. However, the acclimatized mice were no more susceptible to DFP than were the controls that were treated at room temperature (Table I-e).

Discussion. Fuhner(5) and Sanno(6) found that the toxicity of colchicine in frogs was increased 400 to 500 times when the temperature was raised 12°C, *i.e.*, from 20 to 32°C. Also, Hausmann(7) reported that hibernating bats were immune to 30 times the dose of colchicine lethal to non-hibernating bats. Interestingly enough, the lethal dose of colchicine in frogs at 30°C is approximately the same as for mice, about 3 mg/kg(8,9). Earlier work led Jacobj(10) to suggest that the toxicity of the compound was a function of body temperature, the toxic principle being the oxidation product, oxydicolchicine. However, in poikilotherms, body temperature can not be dissociated from the metabolic rate. Mammals, on the other hand, maintain a relatively constant body temperature while their metabolic rates may fluctuate considerably. In the present experiment, colchicine was injected into slightly hypothermic mice whose oxygen consumption was at least twice basal. Under these conditions, the toxicity of the drug was only 0.33 times higher than the controls, as compared to the 400-fold increase in the frogs whose oxygen consumption was doubled (assuming $Q_{10} = 2$) when their body temperature was increased 10°C. This lends further support to the hypothesis that the toxicity (oxidation ?) of colchicine is related to the body temperature rather than to the general metabolic level of the animal.

The increased toxicity of KCN (110%) and Intocostrin (76%) is not surprising in view of the augmented metabolic rate of the mice at 4°C. The lethality of Intocostrin is due to its curare-like action on the muscles of respiration, while KCN inhibits cytochrome oxidase and thus prevents the utilization of oxygen by the tissues. The increased potency of KCN approximated to a large extent the increment in the metabolic rate of the animals in the cold. If the increased oxygen consumption is accompanied by the utilization of

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a greater percentage of the available cytochrome oxidase, it seems logical that lethality in the cold would occur when a proportionately smaller fraction of the total enzyme was inhibited.

Mice that were acclimatized to the cold were no more susceptible to DFP intoxication than were the controls treated at room temperature, while the same drug was 45 per cent more potent in mice that had been exposed to the cold for only 24 hours before injection. The anatomical and functional changes that occur in acclimatization to the cold are not entirely understood. However, several workers have reported significant increases in the kidney and liver weight as a function of long exposure to low ambient temperatures(11,12). These findings, confirmed by recent work done in this laboratory, indicate that the detoxification potential of these tissues in cold-acclimatized animals may be greater than in unacclimatized or normal animals. This phase of toxicology obviously requires more study. However, the generalized decreased resistance of the animals to drugs after short exposure to the cold indicates that these mice were essentially weaker and somewhat hypothermic animals under stress. This is borne out by the relatively high natural mortality evident during the first 24 hours at 4°C.

Summary. The toxicity of colchicine, DFP, Intocostrin and KCN was increased in mice that were exposed to 4°C for 24 hours and maintained in the cold after injection. Animals that were acclimatized to the cold were no more susceptible to DFP than were controls treated at room temperature. The possible factors implicated in these findings are discussed.

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Influence of Histamine upon Fungistatic Action of Antihistaminics. (18548)

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The work of Carson and Campbell(1), which reported the fungistatic action of 3 antihistaminics, prompted an investigation concerning which the following is a preliminary report. In addition to testing fungistasis by several antihistaminics, we were also interested in determining whether the compounds are fungicidal as well. Lastly, it was considered of fundamental biochemical interest to determine whether the antifungal action of antihistaminics is based on interference with utilization of histamine (or a compound or compounds of similar structure) by the fungus.

The compounds studied were as follows: Benadryl, (Diphenhydramine HCl) Parke-Davis; Thephorin, (Phenindamine Tartrate) Hoffmann-La Roche; Thenylene, (Methapyriline HCl) Abbott; Neo-Antergan, (Pyranisamine Maleate) Merck; Pyribenzamine, (Tripelenamine HCl) Ciba, and Diatrine, (Metaphenilene HCl) Wm. R. Warner.* The test culture employed was Trichophyton mentagrophytes (Emmons No. 640).

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