

Hayes *et al.* (3). It is apparent that these Eskimos stored considerably less DDT and DDE than persons in the general population of the United States and somewhat less than meat abstainers. The DDE constituted from 10 to 90 percent and averaged about 73 percent of the total DDT-derived material for the Eskimo. For the general population this percentage was 56 and for meat abstainers, 59. These figures support a view that individuals with a lower degree of exposure to DDT are able to convert DDT to DDE more efficiently. The low storage level is consistent with the low level of the insecticide and its metabolite found in the Eskimo diet in spite of the very high meat content of that diet. The small amount of DDT and DDE that was found in the fat of the native Alaskans is probably accounted for by their limited consumption of imported foods and by their brief intake of hospital food prior to surgery.

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Drug Resistance due to Inbreeding

Abstract. Inbred mice susceptible to audiogenic seizures were found to develop resistance to the protective effects of chlorpromazine and its analogs. It is proposed that the use of such inbred mice provides a unique new tool for studying drug mechanism and drug resistance.

In several earlier reports I have shown that various phenothiazine ataractics exert protection against sound-induced convulsions in mice [audiogenic seizures (1-3)]. There are, however, conflicting reports about the degree of

protection exerted by these agents (4). More recently it was reported that chlorpromazine was totally inactive against convulsions in an inbred strain of Swiss mice (3). The present report is an extension of that preliminary report.

The test chamber and sound source were described in an earlier paper (1). Essentially, the procedure consists of placing groups of five mice in the test chamber and exposing them to auditory stimulation for 1 minute. The criterion used to measure protection in this study was the occurrence or nonoccurrence of convulsions (clonic and tonic).

The diametrically opposed data obtained with chlorpromazine in noninbred and in inbred albino Swiss mice raise the interesting possibility that development of drug resistance may be incidental to inbreeding (3). To investigate this point, careful separation of the various generations of inbred Swiss mice was made, and a systematic bioassay of the effects of chlorpromazine and other agents on each individual generation of inbred Swiss mice was conducted.

The first agent studied under these conditions was chlorpromazine, which had been shown earlier to be a potent antagonist of seizures in noninbred Swiss mice. A constant dose known to be effective in noninbred mice was tested in each separate generation. A summary of the findings is shown in Table 1. The degree of protection exerted by chlorpromazine diminishes from the parental generation to each succeeding generation.

About 91 percent of the P₁ group was protected against convulsions by 10 mg of chlorpromazine. In the F₁ generation, a sharp reduction in protection against convulsions was seen. Only 33.3 percent of the animals were protected. Succeeding generations (F₂, F₃, F₄, and F₅) had protection rates of only 16.3, 7.1, 3.3, and 8.3 percent, respectively. Studies with higher doses (up to 80 mg/kg) did not show any discernible protection.

Control studies of generations F₁, F₂, F₃, F₄, and F₅ showed that these generations exhibited convulsions at a frequency of 90 percent.

Prior to auditory stimulation, all mice used in this research typically exhibited all of the symptoms of chlorpromazine medication: ptosis, heavy sedation, and ataxia. However, auditory stimulation of the "nonprotected" generations re-

Table 1. Protective effect of chlorpromazine on various generations of inbred Swiss mice.

Generation	Number convulsed / number tested	Protection (%)
P ₁	3/34	91.1
F ₁	10/15	33.3
F ₂	41/49	16.3
F ₃	26/28	7.1
F ₄	29/30	3.3
F ₅	11/12	8.3
F ₁₂	10/10	0.0
F ₁₃	10/10	0.0

sulted in immediate arousal followed by wild running that culminated in convulsions. No mice receiving medication were used for inbreeding purposes.

The response of the mice of generations F₁ to F₅ to the auditory stimulus appeared to be a potentiation of a "fright" response, manifested by frenzied running. On the other hand, the proportion of homozygosity to heterozygosity may influence the "strength" of phenotypic expression and drug effect. In addition, the genes may be linked. These are questions to be settled through future research.

Preliminary studies of chlorpromazine analogs (promazine, perphenazine, prochlorperazine, and trifluoperazine) suggest a similar trend. All of these agents have been reported to be effective antagonists of audiogenic seizures in noninbred Swiss mice. However, as shown in Table 2, inbred mice (generations F₂ to F₅) are not significantly protected by these same agents. Thus, a maximal protection against convulsions of only 20 to 30 percent was obtained with promazine, perphenazine, prochlorperazine, and trifluoperazine in the inbred generations. In contrast to the phenothiazine ataractics, the barbiturate sodium phenobarbital uniformly gave complete protection against convulsions in all inbred generations tested.

Table 2. Protective effect of phenothiazine ataractics and phenobarbital on various generations of inbred Swiss mice.

Number convulsed / number tested			
F ₂	F ₃	F ₄	F ₅
<i>Promazine</i>			
5/5	7/10		
<i>Perphenazine</i>			
5/5	5/5	5/5	
<i>Compazine</i>			
	8/10	8/10	
<i>Stelazine</i>			
4/5	4/5	4/5	5/5
<i>Phenobarbital</i>			
0/10	0/10	0/10	0/10

It has been demonstrated that each succeeding generation of inbred mice showed lowered response to the protective effects of chlorpromazine. This finding suggests that genetic characteristics of sensitivity to the effects of chlorpromazine and its analogs are somehow deleted or lost during the course of inbreeding by brother-sister matings. Although this finding is still of a preliminary nature, it may prove possible to investigate neurochemical mechanisms of action of chlorpromazine and its analogs by utilizing differences that may exist between inbred and noninbred mice of the Swiss strain. Thus, it may be possible to find enzymatic intermediates that are deleted by inbreeding and that are essential for normal drug activity. Studies of this nature could lead to a new approach in uncovering key mechanisms of drug action and drug resistance (5).

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References and Notes

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Effect of Meprobamate on the Multiplication of *Brucella abortus* in Monocytes

Abstract. Peritoneal mononuclear phagocytes (monocytes) obtained from guinea pigs that had been treated with meprobamate do not support, in vitro, the intracellular growth of smooth *Brucella abortus* that is characteristic of monocytes from untreated animals. This modification of intracellular events appears to be due to an indirect action of the drug, since meprobamate does not produce any effects following direct exposure of monocytes or bacteria to the drug in vitro. Furthermore, the brucellacidal activity of serum from animals exposed to meprobamate is not increased. An interaction between monocytes and a component in the serum of animals exposed to meprobamate is required for the altered intracellular events.

Virulent organisms of *Brucella abortus* will multiply within peritoneal mononuclear phagocytes (monocytes) of susceptible animals when these cells are maintained in vitro (1); in contrast, monocytes from innately resistant (2),

Table 1. Distribution of brucellae within individual monocytes, and yields of viable brucellae per flask, in cultures initiated with monocytes from guinea pigs exposed to meprobamate (five 100-mg doses over 60 hours) or water. Figures not in parentheses are from experiments in which the drug or water was given orally; figures in parentheses are from experiments involving subcutaneous injections of drug or water.

Age of monocyte cultures*	Monocyte donors treated with	Percentage of monocytes containing indicated number of brucellae†				Viable count per flask‡ (× 10 ⁶)
		0	1-10	11-20	>20	
2	Water	36 (48)	64 (52)	0 (0)	0 (0)	1.41 (0.90)
2	Meprobamate	34 (45)	66 (55)	0 (0)	0 (0)	0.96 (1.20)
24	Water	17 (37)	60 (54)	20 (9)	3 (0)	1.60 (0.95)
24	Meprobamate	42 (44)	54 (53)	3 (3)	1 (0)	1.25 (0.50)
48	Water	10 (17)	22 (38)	5 (7)	63 (38)	35.2 (40.0)
48	Meprobamate	36 (46)	60 (43)	0 (5)	4 (6)	7.45 (1.9)

* In hours following initiation. † Average of counts on two coverslips; 50 monocytes were examined per coverslip. ‡ Average of duplicate counts on each of two flasks.

or from brucella-infected (3), animals support little, if any, intracellular multiplication. In various efforts to find other conditions that might modify intracellular growth in vitro, we found that adrenocortical and gonadal steroids and bacterial endotoxins, when introduced either directly into the tissue culture system or injected into guinea pigs prior to the collection of monocytes, did not affect intracellular growth (4). In a recent study of certain tranquilizers, which in addition to their well-known influence on the central nervous system also have been reported to affect antibody formation (5), resistance to bacterial pathogens (6), and carbon clearance (7), meprobamate had pronounced effects when it was administered to monocyte donors.

Our procedures for harvesting and maintaining monocytes for studies in vitro have been described on several prior occasions (1, 3, 4). Briefly, monocytes were collected 48 hours after intraperitoneal stimulation with saline, introduced into Porter flasks containing 30 percent autologous serum in Hanks' balanced salt solution, and were then exposed to brucellae. Extracellular brucellae were subsequently eliminated by replacing the initial medium with serum-Hanks' solution containing streptomycin (10 µg/ml). Intracellular multiplication was assessed periodically by examining the bacterial contents of individual stained macrophages, and by viable counts on the yields from disrupted monocyte populations. Meprobamate was administered either subcutaneously into the flank, or orally, as five doses (of 50 to 100 mg) over a 60-hour period immediately prior to the collection of monocytes. Control animals received distilled water instead of meprobamate.

When monocyte cultures were initiated with cells from guinea pigs given

meprobamate orally, ingestion was not affected but the intracellular multiplication of virulent brucellae was less in monocytes from meprobamate-treated animals than in monocytes from water-fed controls (Table 1). Similar results were obtained after subcutaneous injection of meprobamate. Since meprobamate has little tranquilizing effect when given by the subcutaneous route, it would seem that the effects observed are independent of the tranquilizing action.

Because meprobamate given orally in these amounts did produce pronounced tranquilization, the effect of the drug on the monocytes might have been a consequence of starvation. However, complete deprivation of food and water for 72 hours did not lead to inhibition of intracellular growth. The drug was not directly bactericidal for brucellae, and sera from meprobamate-treated animals had no more brucellacidal activity than sera collected from the same animals prior to treatment. Further, the addition of meprobamate directly to monocyte cultures did not affect intracellular multiplication of brucellae. Therefore, the effects obtained with monocytes from treated animals must be regarded as the result of an indirect mode of action, possibly involving a metabolite of meprobamate produced in vivo.

To determine whether the in vivo changes that lead to altered properties of the monocytes affected primarily the cells or the serum, the following experiment was performed. Guinea pigs were bled and the serum was stored for later testing. One week later the animals were treated with meprobamate by the oral route, and after termination of treatment another sample of serum was collected and stored. The animals were then allowed to rest for 3 weeks. At this time monocytes were collected and tested for their support of intracellular