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*).

N. PLOTNIKOFF

Stanford Research Institute,
Menlo Park, California

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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*), 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.

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‡ ($\times 10^6$)
		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.

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

multiplication of brucellae in the presence of the previously collected autologous sera. When the pretreatment serum was used, no inhibition of multiplication occurred. However, when monocytes from the same harvest were cultivated in the serum that had been collected immediately after meprobamate treatment, intracellular multiplication was inhibited. Thus it would appear that exposure to meprobamate so alters the serum of treated animals that monocytes cultivated in the presence of this serum no longer allow unrestricted intracellular multiplication of *B. abortus*.

Certain effects of meprobamate on specific enzyme systems are known (*8*). One may therefore hope that the ability of this drug to cause changes in monocytes that lead to properties resembling those of cells from immune animals will permit a better analysis of biochemical changes responsible for so-called cellular immunity (*9*).

R. W. I. KESSEL
JANET BOUGHTON
WERNER BRAUN
Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

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Abscission and Abscisin

In a recent report in *Science* (*1*), the isolation of an abscission-accelerating compound, which the authors called abscisin, was described by Liu and Carns. We feel that, while the report holds some promise for interesting progress, it could be misinterpreted.

First, since the test that is used to assay for the abscission activity is a cotton seedling test which had not been previously reported in the literature, it would have been helpful had Liu and Carns presented more data to illustrate the results. The four values which are given show differences which would be considered very small in the conventional bean explant test, but of course the different plant material may account for the proportionally small differences.

In studying the developmental physiology of plants, when we find a substance with the ability to accentuate a given developmental or metabolic response, we cannot then assume that it serves to accentuate this response *in situ*. Before tentatively concluding that a promotive substance in a plant extract may be involved in a developmental process, we must do more than simply show that it is present. Some correlation of its occurrence with the developmental event is rudimentary to such an implication.

Liu and Carns have extracted something from the brown shells of cotton fruits after maturation and the completion of commercial harvesting. Does the presence of an abscission-promoting substance in this material implicate it in the development of the abscission processes, which would have occurred weeks or even months earlier?

A rather complicated purification procedure is described for the cotton extract. It would have been helpful if, along with this, data had been presented to show that the fractions which were discarded during the purification were without appreciable activity in the abscission test. It is possible that the unused fractions as well as the burs themselves after extraction did in fact include compounds which may be correlated with the abscission process.

A great variety of naturally occurring substances can directly promote abscission, including such classes of compounds as sugars, auxins, amino acids, and unsaturated hydrocarbons. Extraction and purification of any of these substances would surely not warrant the coinage of a new hormonal term and its addition to the literature of plant physiology. To illustrate the great variety of types of compounds which can stimulate abscission, we have assembled the data shown in Table 1. The assay used is the bean petiole explant test, and in each case untreated controls reached 50-percent abscission in approximately 100 hours. These data point up the fact that the abscission process is a complicated product of metabolism, and that under some circumstances it can be promoted by many different types of substances. For comparison, data for some extracts from abscissing and nonabscissing bean leaves are included, in which a tentative correlation with the abscission process can be seen.

Table 1. Promotion of abscission by some widely differing types of substances. The data show the hastening of 50-percent abscission in the bean petiole explant test. The experiments were carried on in light, except for the sucrose experiments, which were in darkness.

Substance	Promotion of abscission (hr)	Reference
Sucrose ($3\times10^{-2}M$)	50	(*2*)
Alanine ($5\times10^{-3}M$)	70	(*3*)
Formaldehyde ($5\times10^{-4}M$)	69	(*3*)
Ethylene (0.01%)	65	(*4*)
Napthaleneacetic acid ($10^{-5}M$)	69	(*5*)
Extract from		
Green leaves*	0	(*4*)
Senescent leaves*	76	(*4*)

* Acetone extracts of bean leaves consisted of dilutions, with water, to ten times the original fresh weight of the tissue extracted.

The concept of control of abscission by hormonal systems other than the auxins is certainly an interesting one, but as yet evidence has not been provided for the existence of other hormones in the sense of chemical messengers controlling the development of abscission.

A. C. LEOPOLD
B. RUBINSTEIN
Horticulture Department, Purdue University, Lafayette, Indiana

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We find it necessary to take issue with Leopold and Rubinstein on a number of points, and further, we wish to present what we believe to be justification for submitting the information on the isolation of abscisin in the form in which it was published.

In such a report there is not space to discuss in detail the many techniques employed. For instance, it seems to us implicit in purification work that discarded fractions are carefully screened for activity before being so-considered. Further, it should not be necessary to state that the accelerated abscission reported was highly reliable and repro-