between *P. tularensis* and *P. novicida* which could be eliminated by diluting either antiserum 1:5. Polyvalent antiserum produced against *P. pseudotuberculosis* stained strains of *P. pestis* deficient in Fraction 1 and species of Salmonella containing either somatic factors 4 and 9. The antigens of various species of the genus *Pasteurella* differ in their reactions with their antibodies after exposure to chemicals used routinely in histological processing. *P. anatipestifer, P. gallinarum, P. haemolytica, P. multocida, P. novicida,* and *P. tularensis* retain their ability to react with species specific antisera after treatment, while *P. pestis* and *P. pseudotuberculosis* are rendered non-reactive by most chemicals used in tissue embedding techniques.

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Antagonism of Anticonvulsants by Adrenergic Blocking Agents. (31111)

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The anticonvulsant effects of diphenylhydantoin(1), acetazolamide(2,3) and chlordiazepoxide(4) have been found to be antagonized by prior administration of reserpine. The mechanism of the antagonism of diphenylhydantoin and chlordiazepoxide by resperpine was found to be by some mechanism other than catecholamine or serotonin depletion(1,4) while that of acetazolamide was found to be mediated through depletion of catecholamines in the central nervous system (2,3). The present study was undertaken to further study the mechanism of action of these anticonvulsants and to determine if their activity was altered by adrenergic blocking agents.

Methods and materials. Male albino mice (Harlan Industries), weighing 18-26 g, were used in all the tests. Maximal electroshock seizures were produced by the method of Swinyard *et al*(5). A current of 50 ma and 0.2 sec duration was delivered *via* corneal electrodes. The criterion for protection against maximal electroshock was abolition of the hind leg extensor component of the seizure.

The ED_{50} values were calculated and compared for significance of differences by the method of Litchfield and Wilcoxon(6). The 95% confidence limits are included in parenthesis in the respective Tables.

The adrenergic blocking agents used in this investigation were: (1) dibenzyline, (2) tolazoline, (3) MJ-1999 (4(2 isopropylamino-

TABLE I. Effect of Adrenergic Blocking Agents on Anticonvulsant Activity of Diphenylhydantoin, Acetazolamide and Chlordiazepoxide.

Treatment	Dose, mg/kg i.p.	ED_{50} diphenylhydantoin, mg/kg i.p.	ED_{50} acetazolamide, mg/kg i.p.	ED_{50} chlordiazepoxide, mg/kg i.p.
Control	—	7.5 (6.4– 8.8)	26.5 (21.5– 33.4)	19.8 (14.7-26.7)
Dibenzyline	3	—	94.0 (74.6–118.4)*	—
	10	—	178.0 (144.7–218.9)*	—
	30	17.8 (14.5–21.9)*	>500*	30.0 (27.4–32.9)*
Tolazoline	30	9.4 (7.0–12.6)	105.0 (91.0–121.0)*	26.3 (23.1–30.0)
MJ-1999	30	8.8 (7.0–11.1)	79.4 (58.8–107.4)*	17.0 (14.2–20.4)
Nethalide	30	9.8 (7.3–13.1)	62.0 (48.9– 78.8)*	11.8 (9.4–14.8)*
DCI	10	7.6 (6.0– 9.3)	15.9 (11.8– 21.5)*	15.2 (12.9–17.9)
Chlorpromazine	3	7.8 (6.6– 9.3)	92.0 (73.5–115.0)*	20.7 (14.7–27.7)

* Probability level $P < .05$.

1-hydroxyethyl) methane-sulfonanilide HCl), (4) nethalide, (5) DCI (1(3′,4′-Dichlorophenyl)-2-isopropylamino-ethanol HCl) and (6) chlorpromazine. In all cases the adrenergic blocking agents were given one hour prior to electroshock and the anticonvulsant was given 30 minutes prior to electroshock. At the doses administered, the adrenergic blocking agents tested were without effect in the maximal electroshock test.

Results. Table I shows the effect of various adrenergic blocking agents on the ED_{50} value of diphenylhydantoin, acetazolamide, and chlordiazepoxide in the maximal electroshock test. The anticonvulsant activity of diphenylhydantoin was significantly decreased by dibenzyline pretreatment. None of the other adrenergic blocking agents tested altered the ED_{50} value of dephenylhydantoin.

Acetazolamide was antagonized by all the adrenergic blocking agents except DCI. The most pronounced antagonism was produced by dibenzyline. Doses as low as 3 mg/kg of dibenzyline antagonized the anticonvulsant effect of acetazolamide while 30 mg/kg of dibenzyline increased the ED_{50} value to greater than 500 mg/kg. The antagonism of acetazolamide by dibenzyline was not the result of an inhibition of absorption since intravenous doses of acetazolamide were also antagonized (not shown). The effect of DCI on the anticonvulsant activity of acetazolamide was of interest since it actually increased the effect of acetazolamide in the maximal electroshock test.

The effect of the adrenergic blocking agents tested on the anticonvulsant effect of chlordiazepoxide was similar to that on diphenylhydantoin. The anticonvulsant effect of chlordiazepoxide was significantly antagonized by dibenzyline.

Discussion. The finding that both alpha and beta adrenergic blocking agents antagonize the anticonvulsant effect of acetazolamide supports the hypothesis that its activity is mediated through a catecholamine mechanism. Previous work(2,3) has shown that the activity of acetazolamide is antagonized by amine-depleting agents such as reserpine, α-methyl tyrosine and several benzoquinolizine derivatives. The effect of reserpine in these cases could be reversed by the administration of 3,4 dihydroxyphenylalanine or prevented by prior administration of monoamine oxidase inhibitors.

The failure of adrenergic blocking agents, with the exception of dibenzyline, to antagonize the anticonvulsant effects of diphenylhydantoin and chlordiazepoxide is also in accordance with the previous findings(1,4) on the mechanism of the anticonvulsant activity of these compounds.

The implication of catecholamines in the mediation of the anticonvulsant effect of acetazolamide may make it a useful tool in studying central adrenergic mechanism. As well, the blockade of the anticonvulsant effect of acetazolamide by both alpha and beta adrenergic blocking agents may lend evidence to the presence of these types of receptor sites in the central nervous system.

Summary. Alpha and beta adrenergic receptor blocking agents were tested for their ability to block the anticonvulsant effect of

diphenylhydantoin, acetazolamide and chlordiazepoxide. The anticonvulsant effect of acetazolamide was significantly antagonized by all the blocking agents but DCI, while the anticonvulsant activity of diphenylhydantoin and chlordiazepoxide was only antagonized by dibenzyline. The blockade of the anticonvulsant effect of acetazolamide by adrenergic blocking agents supports the hypothesis that catecholamines are involved in the anticonvulsant effect of this compound.

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Antiviral Activity of Higher Plants on Lymphocytic Choriomeningitis Infection *in vitro* and *in vivo*. (31112)

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Limited success in antiviral chemotherapy with drugs of synthetic origin at the animal level has led us to search in the field of natural products. In addition to 6 higher plants which were found to be active against Columbia SK virus in mice(1), we have now found 2 more higher plants in a group of 180 different Chinese medicinal agents, mostly herbs, remarkably active against lymphocytic choriomeningitis (LCM) infection. In contrast to the herbs, out of several hundred synthetic compounds, only 3 showed *in vitro* activity, and none showed *in vivo* activity.

Materials and methods. 1. *Herbs.* About 180 Chinese herbs, selected because of traditional anti-inflammatory or anti-tumor effects, were collected or purchased from the Pacific-Asian area. The plants, portions of plants, or other material were extracted by boiling in water for 30 minutes. The supernatants after centrifugation were concentrated at 60°C to represent a 40% solution of the original weight of herb, and then kept at 4°C until use.

2. *Defined chemical compounds.* Two compounds, α-hydroxybenzyl-benzimidazole (HB-B) and 5-methyl-tryptophan (Me-TP), which we reported to have anti-LCM activity *in vitro*(2), and several hundred synthetic chemicals were dissolved or suspended in distilled water and kept in a freezer until used.

3. In vitro *test.* Use was made of a strain of LCM virus adapted from the original NY-621 strain to KB cells in stationary culture maintained in Medium 199 with 5% calf serum(2,3). Drugs were added to 3-day-old cultures immediately after inoculation of 10 $TCID_{100}$ of the virus. Drugs were designated as effective when the viral cytopathic effect (CPE) showed only 0, 1+ or 2+ damage, in comparison with 4+ in the controls, after 2 days' incubation. The maximum concentration during 10 days of incubation, as compared with controls, was designated as the maximum non-toxic dose (MNTD), and the MNTD was used for the antiviral screening test. Virus titers of harvests from cultures which showed definite anti-CPE activity were also measured to ascertain the degree of activity of the drugs.

4. In vivo *test.* Drugs were injected subcutaneously to 9 to 12 g mice in a dose of 1 MNTD 2 hours after intracerebral inoculation of LD_{80-90} of LCM virus (Strain NY-621); injections were continued twice daily for 5 days. Observation was continued for an additional 9 days. At MNTD, mice showed no toxic signs during the observation period. Ten treated mice and 10 control mice were used in each experiment. Substances appearing to be active were tried repeatedly.

Results. 1. *Antiviral activity against LCM*