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*

virus in KB cells. About 150 synthetic chemicals and 180 kinds of Chinese herbs have been tested against LCM infection in KB cells since our last paper was published(2). Of these 330 preparations, only one synthetic chemical (NSC47594; benzaldehyde, 4-hy-

TABLE I. Inhibitory Effect of Drugs on CPE and Propagation of LCM Virus in KB Cells.

	MNTD, mg	Dilution of drugs from MNTD 1:2 CPE	1:2 TCID	1:4 CPE	1:4 TCID	1:8 CPE	1:8 TCID
HBB	.1	0	3*	2+	5	4+	7
Me-TP	.4	0	3	2+	5	4+	7
NSC47594	.1	0	3	0	3	2+	5	4+	7
H6512	1.	2+	6	4+	7		
H6727	.6	0	3	0	3	2+	5	4+	7
" —autoclaved	.6	0	3	0	3	2+		4+	

CPE = cytopathic effect; TCID = tissue culture infective dose; MNTD = maximum non-toxic dose.

* Indicates 10^3 $TCID_{100}$ of harvest 2 days after inoculation of 10 $TCID_{100}$ of LCM virus.

TABLE II. Antiviral Activity of Herbs and Synthetic Chemicals on LCM Infection in Mice.

Drug	MNTD, mg	Treated, Died/Total	Control, Died/Total	Treated / Control
H6727	5.	4/10	7/10	
"	5.	5/10	9/10	
"	5.	4/10	9/10	
"	5.	5/10	9/10	
"	5.	4/10	9/10	
"	5.	4/10	9/10	
"	5.	3/10	7/10	
"	5.	3/10	9/10	
"	5.	1/10	9/10	
	total	33/90 (37%)	77/90 (86%)	43%
H6512	40.	3/10	9/10	
"	40.	3/10	9/10	
"	40.	5/10	9/10	
"	40.	5/10	9/10	
"	40.	5/10	9/10	
"	40.	3/10	8/10	
"	40.	4/10	8/10	
"	40.	4/10	10/10	
"	40.	2/10	9/10	
"	40.	4/10	9/10	
"	40.	2/10	9/10	
	total	40/110 (36%)	98/110 (89%)	41%
H6727—autoclaved	5.	5/10	9/10	
" "	5.	6/20	8/10	
" "	5.	3/10	9/10	
" "	5.	5/10	9/10	
" "	5.	4/10	9/10	
	total	23/60 (36%)	44/50 (89%)	52%
H6512—autoclaved	40.	5/10	9/10	
" "	40.	5/10	8/10	
" "	40.	2/10	7/10	
	total	12/30 (40%)	24/30 (80%)	50%
NSC47594	1.	8/10	7/10	100%
"	1.	8/10	9/10	89%
HBB	2.	10/10	9/10	100%
"	2.	9/10	9/10	100%
5-methyltryptophan	4.	10/10	9/10	100%
"	4.	8/10	9/10	89%

droxy-3, 5-diiodo-, oxime) and 2 higher plants (H6512; *Magnolia kobus,* DC. and H6727; *Narcissus tazetta* L.) were found to be effective. Comparison of the activity of these with HBB and 5-methyl-tryptophan(2) is shown in Table I; all 3 were more active than the previous compounds. H6727 was the most active, and it was noted that it was not destroyed by heat (120°C, 15 min).

2. *Antiviral activity against LCM infection in mice.* The 5 drugs active *in vitro* (Table I) were then tested in mice as shown in Table II. It was found that the 2 herbs showed remarkable activity, while the 3 synthetic chemicals were not effective. It was of interest that H6512 showed strong activity in mice in spite of the weak activity in tissue culture. Autoclaving again did not destroy activity.

Discussion. LCM virus is one of the causative agents (3 to 5%) of aseptic meningitis in man, and several surveys of trapped wild mice indicate that up to 20% harbor the virus and excrete it in their urine(4). Neutralizing antibodies for the virus have been demonstrated in the blood of about 12% of normal people in the United States indicating widespread subclinical infection(5). Preparations made from 2 Chinese herbs have been shown to have activity against this virus in mice, while several other substances with *in vitro* activity have been found to be ineffective *in vivo.*

Further studies will be done against other neurotropic viruses at the animal level, and chemical purification of the active principles carried out. At present, it has been demonstrated only that the active principles of both herbs are water-soluble and heat-resistant. Preliminary experiments suggested that the antiviral action of both herbs was probably at the intracellular level because they did not inactivate the virus when mixed with it in tissue culture medium without cells and incubated for 2 hours at 37°C or overnight at 4°C.

In spite of showing strong activity in mice, H6512 showed only weak activity in tissue culture. Two possible explanations are suggested. The crude extracts may contain substances toxic to tissue culture cells, precluding observation at high concentrations, while mice may be more resistant to the toxic effect; in this case purer extracts may exhibit stronger *in vitro* activity. Alternatively, substances not active *in vitro* may be changed to active derivatives in intact mice.

Summary. Two higher plants (*Magnolia kobus* DC. and *Narcissus tazetta* L.) have emerged from a screening program of Chinese medicinal agents as antiviral drugs against lymphocytic choriomeningitis infection in mice.

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