specimens of urine varied from 0.14 to 3.75 μg/6 hr/m^2 during zero AD titer periods and between 0 and 6.36 μg/6 hr/m^2 when AR titers were elevated. The data indicate that aldosterone secretion continued in the presence of elevated antibody titers despite neutralization of renin pressor response. The sodium diuresis which occurred in the presence of elevated AR without alteration in K excretion does not appear to correlate with depressed aldosterone formation and the causative factor modifying electrolyte excretion remains uncertain.

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Antiviral Activity of Herbs on Columbia SK in Mice, and LSM, Vaccinia and Adeno Type 12 Viruses *in vitro*.* (30527)

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The difficulty in finding active therapeutic drugs of synthetic origin for experimental virus infections in animals has led us to search in the field of natural products. In addition to propionin, an unidentified antiviral substance extracted from *Propionibacterium freudenreichii*(1,2), we have found 6 higher plants that show antiviral effects against Columbia SK virus infection in mice. Also, 24 plants have been found to show antivaccinia effects in tissue cultures.

Materials and methods. About 130 kinds of herbs, constituting the bulk of the agents commonly used in Chinese medicine in the Pacific-Asian area, were collected or purchased. The plants or portions of plants were extracted by boiling in distilled water for 30

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TABLE I. Antiviral Activity of Herbs on Vaccinia, LCM and Adeno Type 12 Viruses in KB Cell Cultures.*

	Name of herb	MNTD† (mg)	Cytopathic effect (CPE) Vaccinia (100 TCID)	LCM (10 TCID)	Adeno 12 (100 TCID)
N	*Angelica sinensis*	.1	2+	4+	4+
S	*Lashiosphaera fenzli* keich	10.	2+	4+	4+
H6507	*Portulaca oleracea* L.	4.	2+	4+	4+
H6511	*Plantago major* L.	2.	2+	4+	4+
H6512	*Magnolia kobus*, DC.	1.	2+	2+	4+
H6513	*Evodia rutaecarpa*	1.	1+	4+	4+
H6514	Squash seeds	10.	2+	4+	4+
H6515	*Gardenia florida* L.	2.5	1+	4+	4+
H6519	*Bupleurum falcatum* L.	.6	1+	4+	4+
H6520	*Cimicifuga foetida*	.6	2+	4+	4+
H6525	Rabbit feces	5.	2+	4+	4+
H6527	*Siler divaricatum* benth, hook	2.5	4+	4+	4+
H6532	*Agrimonia aeupatoria*	10.	4+	4+	4+
H6541	*Cyrtomium fortunei* J. Sm.	2.5	1+	4+	4+
H6542	Sacred O Lelo Berries	2.5	1+	4+	4+
H6551	*Dolichos lablab*, L.	10.	1+	4+	4+
H6556	*Deucaena glauca* (Koa Haole)	2.5	2+	4+	4+
H6557	*Eriocaulon sieboldianum* S.Z.	10.	1+	4+	4+
H6561	*Elscholtzie patrini* garcke.	2.5	2+	4+	4+
H6589	*Datura inermis* jacq.	2.5	1+	4+	4+
H6594	*Cocculus thunbergii* DC.	10.	1+	4+	4+
H6595	*Curcuma aromatica* salisb.	2.5	1+	4+	4+
H6701	*Pteropus pselaphon* lay feces	10.	2+	4+	4+
H6704	*Hedyotis diffusa* willd.	2.5	2+	4+	4+
H6706	*Liquidambar formosana* hance	10.	1+	4+	4+
H6709	*Vaccaria pyramidata* merik	10.	1+	4+	4+
Control	(Without herb)		4+	4+	4+

* Drugs were added just after inoculation of viruses; medium including drugs was changed every 2 days. The degrees of CPE were recorded on the day when controls showed maximum (4+) changes.

† Maximum non-toxic dose.

minutes. The supernatants after centrifugation were concentrated to 40% solutions by heat (60°C) and then kept at 4°C until use. *In vitro* tests were made against vaccinia (IHD), LCM (NY621 strain)(4), and adeno type 12 (Huie) viruses in KB cells in stationary cultures maintained in Medium 199 with 5% calf serum. Solutions from herbs were added to 3-day-old cultures immediately after inoculation of 10 to 100 $TCID_{100}$ of the virus. Herbs were designated as effective when the viral cytopathic effects (CPE) showed 0, 1+ or 2+, in comparison with 4+ in controls. The maximum concentration of herbs which allowed monolayers of cells to remain in good condition during 10 days of incubation, as compared with controls, was designated as maximum non-toxic dose (MNTD), and one-half of MNTD was used for the antiviral screening tests. The medium containing the herb solution was changed every 2 days.

In the *in vivo* experiments with Columbia SK virus, maximum non-toxic doses (MNTD) of herbs were injected subcutaneously 2 hours after intraperitoneal inoculations of LD_{70-90} of the neurotropic, RNA virus, and continued twice a day for 5 days. Observation was continued for an additional 5 days. At MNTD mice showed no toxic signs during the observation period. Ten treated mice and 40 control mice were used in each experiment. Some herbs were also tested for antiviral activity in the system of Columbia SK virus and Ehrlich ascites tumor cells *in vitro* as reported before(3).

Results. 1. Antiviral effects of herbs on vaccinia, LCM and adeno type 12 viruses in KB cells. About 130 herbs were treated. It was found that 24 showed antivaccinia effects, one (H6512) showed activity on LCM, and none was active against adeno type 12 virus. Table I shows the names of the herbs and the degree of their antiviral

TABLE II. Antiviral Activity of Herbs on Columbia SK Infection in Mice; Comparison with Propionin.

Herb	MNTD* (dry wt)	No. of mice: Treated Died/Total	%	Control Died/Total	%	Treated/Control %
H6507	80 mg	20/50	(58)	195/200	(80)	73
H6513	80 "	48/80	(60)	244/320	(76)	77
H6514	80 "	41/70	(59)	217/280	(78)	76
H6527	40 "	27/50	(54)	160/200	(80)	68
H6532	120 "	28/50	(56)	154/200	(77)	71
H6551	40 "	39/70	(56)	218/280	(78)	72
Propionin (partly purified)	.1 ml	101/290	(35)	784/960	(82)	43
Propionin† (crude) (tested in 1961 and 1962)	.025 "	377/745	(51)	952/1310	(73)	70

* Maximum non-toxic dose.

† Included for comparison(2).

activity.

2. Antiviral effects of herbs on Columbia SK infection in mice. Of 90 herbs tested, 6 herbs (H6507, 6513, 6514, 6527, 6532 and 6551) were found to be repeatedly (5 to 8 times) effective. Table II shows the results. The 6 herbs decreased mortality from the virus infection to about three-fourths that in the controls. This effect was of the same degree as previously reported for crude propionin(2), but less than that from partially purified propionin tested at the same time. Of the 6 herbs, only 4 were active *in vitro*, using the system of Columbia SK virus and Ehrlich ascites tumor cells with the same method reported before(5,6).

Discussion. Many compounds showing antiviral effects in tissue culture systems have been reported by numbers of investigators, but very few have shown therapeutic value against experimental virus infections in animals(7). In the last 26 years in this laboratory, many selected chemicals have been tested against Columbia SK and ectromelia viruses in mice. Of these the most potent against Columbia SK infection has been propionin. We now report several hopeful drugs from Chinese medicine relatively as effective as crude propionin in mice.

In spite of showing activity in mice, 2 herbs (H6513, 6514) did not show detectable activity *in vitro*. Three reasons might be responsible for this dissociation. Toxic substances against Ehrlich tumor cells in these crude extracts of herbs might preclude observation of activity, while mice might be more resistant to the toxic effects. Substances which were not active *in vitro* could be changed to active ones in mice. Herbs could increase general resistance to virus infection by hormonal or other changes. If it is due to the first reason, purification of the material may remove the toxic substance and allow demonstration of *in vitro* effect. From the present and previous(6,8,9) experiments in this laboratory, it is suggested that vaccinia is generally more susceptible to drugs than other viruses in tissue culture systems.

Summary. About 130 Chinese herbs have been screened for antiviral effect *in vitro* or *in vivo*. Crude water extracts of 6 herbs out of 90 tested *in vivo* decreased the mortality to three-fourths of that in controls, in mice infected with Columbia SK virus. These antiviral activities were comparable with that of crude propionin which has been extensively studied in this laboratory. It was also found that 24 herbs showed activity against vaccinia virus, one herb against LCM virus, but none against adeno type 12 virus in KB cell cultures.

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Effect of Hyperimmune Rabbit Serum on Intracellular Growth of *Mycobacterium bovis.** (30528)

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Experimental evidence of humoral immunity in tuberculosis is inconclusive(1,2). Although the presence of a phagocytosis-promoting factor in sera from tuberculous animals(3) and patients(4) has been demonstrated, opsonization as a positive role in resistance to tuberculosis is controversial(5). Fong *et al*(6) reported that immune serum increased the resistance of immune histiocytes to the toxic effect of virulent tubercle bacilli. However, this resistance factor was nonspecific. Recently, Hsu(7) found that, although the growth of *M. tuberculosis* in macrophages of susceptible rabbits was not suppressed in the presence of anti-BCG serum, the parasitized cells were somewhat protected from the damaging effect of the contained bacilli.

Reports by Shepard(8) and from our laboratory(9) indicated that established cell lines may be useful in studying the host-parasite relationship in tuberculosis. As part of a study of the mechanism of resistance and immunity to tuberculosis, experiments were carried out on the influence of hyperimmune anti-BCG serum on (a) phagocytosis of virulent *Mycobacterium bovis* by the J-111 cell line of human leukemic monocytes(10), (b) growth of the phagocytosed mycobacteria and (c) survival of infected cells.

Materials and methods. For hyperimmune anti-BCG serum, commercial New Zealand albino rabbits were given 3 consecutive monthly intravenous injections of 20×10^6 to 40×10^6 viable units of BCG-IV (Rosenthal)† as described by Fong *et al*(11). BCG agglutinins were observed at a serum dilution of 1:640. Antityphoid serum was obtained from animals one week after an immunization regimen described by Fong *et al* (6). Agglutination titers against typhoid O and H antigens were 1:1280 or greater. Uninjected rabbits served as the normal serum source. All sera were stored at —10°C. Where indicated, sera were inactivated at 56°C for 30 minutes.

Stock cultures of J-111 cells were maintained on a yeast extract-rabbit serum medium containing 50 units of penicillin (PN) and 50 μg of streptomycin (SM) per ml. Cells, trypsinized with 0.25% trypsin, were suspended in the growth medium to yield 45,000 cells per ml. One ml volumes were pipetted onto coverslips in Leighton tubes and cultures were incubated at 37°C for 48 hours. Two washings with Hanks' Balanced Salt Solution (BSS) were followed with refeeding of yeast extract medium containing the appropriate test serum in 10% concentration. Infected cell cultures were inoculated with 1.2×10^6 to 1.5×10^6 viable units of *M. bovis* (Ravenel);‡ noninfected cultures received 0.05 ml of BSS. The mycobacterial suspension was prepared from a 3- to 4-week-old culture grown on Lowenstein-Jensen medium. Using

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