ANTIMALARIAL AND ANTIBACTERIAL SUBSTANCES SEPARATED FROM HIGHER PLANTS[1]

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With the advent of penicillin many workers sought similar agents that were bacteriostatic or bactericidal *in vivo*. Such work has stimulated some investigators to seek like substances in higher plants. Osborn (1943) and Lucas and Lewis (1944) prepared extracts from a large series of plants and found many to contain inhibitory substances which were active against microorganisms *in vitro*. The object of the present study was to determine whether crude and refined extracts prepared from wild and domestic higher plants would inhibit the growth of microorganisms such as malaria parasites, nonpathogenic protozoa, bacteria, fungi, and viruses *in vitro* and *in vivo*.

In an attempt to separate inhibitory substances for malaria parasites, bacteria, and viruses, extracts were prepared from more than 200 wild plants collected in the semiarid region of southeastern Oregon and evaluated. Many of these have been found to contain certain substances which have proved to be bacteriostatic or bactericidal to microorganisms *in vitro*. The work was done without previous knowledge of work which has been published during the last two years.

A few of the genera studied are found throughout the world, such as *Artemisia* (sagebrush) and *Ranunculus* (buttercup). These two plants show a preference for the temperate and cooler regions of the northern hemisphere. Boas (1934) was the first to observe that extracts from species of *Ranunculus* retarded the growth of microorganisms. Further work by Boas and Steude (1935), Keding (1939), Schmidt (1942), Osborn (1943), and Seegal and Holden (1945) has supplied additional evidence that extractions from this family contain bacteriostatic and bactericidal properties against microorganisms. The available literature failed to reveal additional evidence on inhibitory activity of any of the other plants to be reported.

In vivo tests of each plant extract against malarial and bacterial infection would have been time-consuming and expensive without obtaining the desired knowledge. It was therefore decided to screen each plant extract by testing it on the seeded agar plate. If a specific plant extract was found to inhibit the test organism, it was then investigated further by more comprehensive *in vitro* and *in vivo* tests.

The plants to be reported on are buttercup (*Ranunculus occidentalis*, Nutt.),

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sagebrush (*Artemisia tridentata*), mountain pasque (*Anemone occidentalis*), dwarf waterleaf (*Hydrophyllum capitatum*), and juniper (*Juniperus occidentalis*, Nutt.).[5]

Preparation of extracts. Fresh plants were finely ground with refined silica and a mortar and pestle, and then an equal volume of normal saline was added. This crude mixture was strained through two layers of gauze to remove larger particles, the filtrate being placed in the cold room until used. These suspensions will be referred to as crude saline extracts in the text and will be designated by the number C10 following the plant number, such as, buttercup extract P16C10.

TABLE 1

Plant preparations

Buttercup	
P16C10	Saline extract
P16B69	Volatile oil
P16B90	Na salt
Sagebrush	
P13C10	Saline extract
P13C10a	Saline extract concentrate
P13B24	Acetone-insoluble fraction
P13B31	Acetone-soluble gum
Dwarf Waterleaf	
P24C10	Saline extract
P24C10a	Saline extract concentrate
P24B1	Alcoholic extract
Mountain Pasque	
P158C10	Saline extract
P158B1	Steam distillate
P158B2	Volatile oil
P158B3	Ether-insoluble fraction of P158B1
Juniper	
P14C10	Saline extract
P14B1	Alcoholic extract
P14B2	Alcohol-insoluble, water-soluble fraction

Extracts with extreme pH levels were adjusted to 7.0. In the preparation of the refined extracts the following methods were followed in each case (table 1):

Buttercup extract P16B69. The extract was an oil obtained from ether extraction of the sodium-chloride-saturated steam distillate of the plant. The oil obtained was diluted 1:10 in absolute alcohol, and this was added to saline to obtain a final concentration of 1:400.

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Buttercup extract P16B90. The same procedure was followed through the ether extraction of P16B69. The ether extract was vigorously shaken with anhydrous Na_2SO_4 until the ether was dry. Metallic sodium was added to the dry ether. A brown precipitate was obtained. This precipitate was washed eight times with ethyl alcohol. The resulting substance was water-soluble and was designated as P16B90.

Sagebrush extract P13B24. The green portions of the plant were chopped and placed in diethylene dioxide. This mixture was incubated at 56 C for 3 to 4 days. The plant residue was filtered off, and the pungent dark green solution was concentrated *in vacuo* to a thick aromatic syrup. This syrup was boiled with 20 volumes of water for 5 minutes and filtered while hot. The chlorophyll was removed in the form of a resinous gum, which remained on the filter paper. The filtrate, tan in color and colloidal in appearance, was concentrated *in vacuo* again to a thick syrup or until foaming ceased. This residue was frozen with CO_2 ice, removed from the tube, and exposed to air for 12 to 18 hours. The brown gum obtained was extracted with acetone. This extraction yielded a yellow precipitate which was washed four times with acetone to remove the last traces of gum. This precipitate was water-soluble and designated as P13B24.

Sagebrush extract P13B31. The acetone-soluble fraction from the preceding extraction was evaporated to dryness *in vacuo*. The residue was a thick, aromatic, water-insoluble gum. This extract was made by dissolving the gum in alcohol and dispersing this solution in water.

Juniper extract P14B1. The berries of this plant were extracted with alcohol for 24 hours, and the berries were removed by filtration. The filtrate was evaporated to dryness. The residue was boiled with water, and the resulting colloidal suspension was designated as P14B1. Extract P14B2 was made by boiling the berries with water after the alcoholic extraction.

Waterleaf extract P24B1. The plant was soaked in normal saline solution for 24 hours at 8 C, filtered, and the residue extracted with alcohol for 30 days. The first 7 days the temperature was maintained at 37 C and thereafter at 8 C. The alcoholic extract was filtered and evaporated to dryness; the resulting residue was suspended in water and designated as P24B1. The plant concentrate was prepared by evaporating the saline suspension *in vacuo* at 45 C. The solution was concentrated to approximately 0.1 volume, and is known as P24C10a.

Mountain pasque extract P158B1. This extract was prepared by steam distillation of the plant. Ether extraction of the distillate and evaporation of the extract leave an acrid oil, which was designated P158B2. P158B3 was the aqueous solution after ether extraction.

In vitro tests. In the screening test used for each plant extract the Oxford cup method was used. One ml of an 18-hour culture of a recently isolated *Staphylococcus aureus* (hemolytic) was used to seed 100 ml of nutrient agar. After the shake cultures were added to the petri dishes, the agar surface was allowed to dry for 30 minutes. Sterile porcelain cylinders were placed on the surface of the seeded agar and filled with plant extracts. The plates were incubated at 37 C for 24 hours. Zones of partial or complete inhibition of growth of the organisms

were measured in cm. When comprehensive tests were indicated, the same procedure was followed using different microorganisms. They included the molds, *Penicillium oxalicum*, *Penicillium notatum*, *Aspergillus* sp., and *Mucor* sp.; bacteria, *Corynebacterium diphtheriae*, *Pseudomonas aeruginosa*, *Eberthella typhosa*, *Clostridium sporogenes*, and *Streptococcus viridans;* and soil organisms, *Micromonospora* (Waksman), *Micromonospora* sp., and five species of *Streptomyces*.

Vapors from the various extracts were tested for their inhibitory activity by placing six drops of the extracts in the top of an inverted seeded agar plate prepared as described above. The cultures were incubated at 37 C for 24 hours. Zones of inhibition of growth were measured in cm. Activity for greater distances was obtained by placing a seeded agar plate over the top of a glass cylinder in which extracts were placed at the bottom. To remove any possibility that the liquids were being transferred by capillary action, a seeded plate was suspended in a bell jar having a diameter of 12 inches. The jar was incubated, and the inhibition zones were measured in 24 hours.

Dilution series were prepared of several extracts to ascertain their bacteriostatic and bactericidal characteristics. Varying amounts (1:1 to $1:2 \times 10^4$) of the extracts were added to nutrient broth, which was then seeded with the test organisms. The tubes were incubated at 37 C for 4 and 24 hours. Samples were taken after incubation and streaked on blood agar plates.

In vitro action of plant extracts against protozoa was determined by placing the organisms in the presence of the plant extract. Malaria-parasitized blood (*Plasmodium gallinaceum*) was mixed with extracts 1:1 and incubated at room temperature (25 to 29 C) for 6 hours. The blood was so diluted that it contained 8×10^6 parasitized cells per ml. One million of the treated cells were inoculated intravenously in 2-week-old chicks. These chickens were observed for 4 weeks, with blood smears being taken every other day.

Clone cultures of fresh-water protozoa (*Paramecium multinucleatum*, *Tetrahymena gellei*, and *Euglena* sp.) were added to 10 tubes, 1 ml each. To the first tube 1 ml of the saline extract of buttercup was added, 0.9 ml to the second tube, 0.8 to the third tube, and so on to the tenth tube to which 0.1 ml of the extract was added. The tubes were observed every 10 minutes for 1 hour.

Toxicity tests. Chickens: Four 2-week-old chicks were given 0.5 and 1 ml of the plant extracts by the intraperitoneal, intravenous, or subcutaneous route. The birds were observed 10 and 60 minutes and 24 hours after receiving the extracts. *Mouse:* Four 4-week-old white Swiss mice were inoculated with 0.25, 0.5, and 1.0 ml intraperitoneally and 0.5 ml subcutaneously of the plant extracts. The animals were observed at 10 and 60 minutes and at 24 hours after injection.

Chronic toxicity reactions were observed in those animals on test during prolonged treatment periods.

In vivo tests. The inhibitory action of the plant extracts on the malaria parasite was tried in the chicken. Three 2-week-old healthy chicks were injected intraperitoneally or subcutaneously with the extracts, 0.5 ml twice daily. The extract was started 2 days before infection with the malaria parasite. The infective dose of *Plasmodium gallinaceum* was 10^6 parasitized cells. Blood smears

were taken every other day after the fourth to fifth day of inoculation. Birds succumbing after the ninth day of the disease were also observed for exoerythrocytic forms of the parasite in brain smears.

White Swiss mice, 4 weeks old, were inoculated intraperitoneally with 0.5 ml of a 6-hour broth culture of *Diplococcus pneumoniae* (type 19). One hour after inoculation with the test organism the mice were given intraperitoneal injections of the plant extracts. Thereafter the extract was given twice daily in predetermined doses. Animals dying in the test and control groups were autopsied

TABLE 2

Effect of extracts of plants on Staphylococcus aureus by the Oxford cup method

Plant extracts	Inhibition-diffusion zones in cm (diameter)
Buttercup	
P16C10	3.5–9.0
P16B69	5.0*–9.0
P16B90	2.3
Sagebrush	
P13C10	1.8
P13B24	2.0
P13B31	1.3
Juniper	
P14C10	3.2
P14B1	†
P14B2	†
Waterleaf	
P24C10	1.0
P24B1	2.3
Mountain Pasque	
P158C10	3.5*
P158B3	†
P158B2	9.0

* Partial inhibition beyond recorded zone of complete inhibition.

† Inhibition in cup area only.

and cultures taken. The degree of infection was determined by comparison with the untreated control infections.

RESULTS

In vitro activity of plant extracts. Table 2 summarizes the results obtained using extracts of five plants against *Staphylococcus aureus* by the Oxford cup method. Saline extracts of the five plants were found to inhibit *Staphylococcus*. The extracts P14B1 and P14B2 of juniper and P158B1 of mountain pasque contained no diffusible antibacterial properties when tested in this manner. Buttercup extracts P16C10 and P16B69 and P158B2 extract of mountain pasque were

found to inhibit completely the growth of all organisms in the plate. In later experiments this action was found to be due to a vapor. Waterleaf extract P24B1 was found to contain substances which were bacteriostatic. Later the same substance was found to have good anesthetic qualities *in vivo* (see Toxicity). P13B31 extract of sagebrush was found to have similar characteristics but not as pronounced.

Complete inhibition of all growth of the *Staphylococcus* in the seeded agar plates by extracts P16C10 and P16B69 of buttercup and P158B2 of mountain pasque—the Oxford cup method being used—indicated that there was good diffusion of the active principle, or that the inhibitory activity was due to the presence of vapor which was soluble. To ascertain the origin of this specific activity of the extracts, a small amount of the extracts was placed in the seeded petri plate without being in direct contact with the agar surface. These extracts were placed in the bottom of the inverted petri plate cover and after incubation at 37 C for 24 hours were observed to have no visible growth. To test the vapors' activity at greater distances than $\frac{3}{4}$ inch, seeded *Staphylococcus* plates were placed over the open tops of glass cylinders in which 6 to 10 drops of the extracts had been placed in the bottom. After incubation the plates were observed to contain no growth of the test organism in the exposed areas. Distances of from $\frac{3}{4}$ to 20 inches were used. As a control against capillary action of the liquids used, a seeded petri plate was suspenced in a bell jar 12 inches in diameter. A small amount of the buttercup extract P16C10 was placed in the bottom. The bell jar was incubated at 37 C for 18 hours. No growth occurred, indicating that the inhibitory action was due to the vapor. By extraction of the steam distillate of both plants, buttercup and mountain pasque, with ethyl ether and evaporation an oil was obtained. The oil exhibited the same properties as the saline extract when tested by the vapor method. The oil when placed in direct contact with the seeded agar inhibited the growth of the test organism, *Staphylococcus aureus*, in 5.0-cm diffusion areas.

The saline extract P16C10 and the vapor of buttercup were tested against *Penicillium* and *Aspergillus* species of molds, *Micromonospora* (Waksman), 5 species of *Streptomyces*, *Eberthella typhosa*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus viridans*. The methods described for the screening tests were used. The growth of all organisms was completely inhibited by the action of the vapor in the exposed areas. The activity of the extract produced inhibition-diffusion zones ranging from 3 to 5 cm on gram-positive organisms and 9.0 cm or complete inhibition on gram-negative organisms and molds.

The bacteriostatic and bactericidal activity of the P16C10 extract of buttercup is summarized in table 3. From the results of the experiments, the inhibitory effect of the saline extract appears to be bactericidal for gram-negative organisms and bacteriostatic for gram-positive organisms. Dilutions higher than 1:5,400 of the crude saline extract failed to inhibit the growth of gram-negative organisms. Concentration or purification of the active principle undoubtedly would greatly increase the dilution factor.

TABLE 3

Bactericidal and bacteriostatic effect of buttercup extract P16C10 on gram-negative and gram-positive bacteria

TUBE NO.	EXTRACT DILUTION	BACTERIA													EXTRACT CONTROL	
		S. aureus		*E. typhosa*		*C. diphtheriae*		*P. aeruginosa*		*S. viridans*		*D. pneumoniae*		*C. sporogenes*[3]		
		1	2	1	2	1	2	1	2	1	2	1	2	1	1	2
1	1:1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	1:3	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
3	1:5	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−
4	1:9	−	++	−	−	−	−	−	−	−	+	−	+	−	−	−
5	1:160	+	+++	−	−	−	−	−	−	−	++	−	++	−	−	−
6	1:320	+	+++	−	−	+	++	−	−	+	++	+	++	++	−	−
7	1:680	+	+++	−	−	+	+++	−	−	+	+++	+	+++	++	−	−
8	1:1,360	+	+++	−	−	+	+++	−	−						−	−
9	1:2,720	+	+++	−	−	+	+++	−	−						−	−
10	1:5,440	++	+++	−	+	+	+++	+	−						−	−
11	1:10,000	++	+++	++	+++	++	+++	++	+++						−	−
12	1:20,000	++++	++++	++++	++++	++++	++++	++++	++++						−	−
13	Organism Control	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	−	−

−, no growth; +, slight; ++, fair; +++, moderate; ++++, profuse.

1. Visual and microscopic appearance.
2. Growth on blood agar—4 mm loop.
3. Agar tubes.

The inhibitory action of the plant extracts against *P. gallinaceum* was tested by mixing 1:1 the extract and parasitized blood, previously diluted so that each ml contained 8×10^6 parasites. These mixtures were incubated at room temperature for 6 hours. One-quarter ml (10^6 parasitized cells) of the mixture was given intravenously to each chick. Three 2-week-old chicks were used in each test. The results of the experiment are presented in table 4. All the substances tested in this manner were lethal to the parasite. The control animals succumbed to malaria infections after receiving parasites treated in a similar manner with saline. The pH of each extract was adjusted to approximately 7.

Fresh-water protozoa (*Paramecium multinucleatum*, *Tetrahymena gellei*, and *Euglena* sp.) in clone cultures were found to lose their motility when placed in varying amounts of the saline extract P16C10 (buttercup). *Euglena* was found to lose its motility in less than 10 minutes in all tubes. *Paramecium* lost activity

TABLE 4

In vitro effect of saline extracts of plants on Plasmodium gallinaceum

PLANT EXTRACT	NO. CHICKS	HOURS INCUB.	DIED	BLOOD POS.	DAYS OBSERV.	CONTROLS		EXO. FORMS	
						Blood pos.	Died	Test	Controls
Buttercup									
P16C10	3	6	0	0	40	3	3	0	0
P16C10b*	3	6	0	0	40	3	3	0	
Mountain pasque									
P158C10	3	6	1	0	33	3	3	0	1
Waterleaf									
P24C10	3	6	0	0	30	3	3	0	0
Sagebrush									
P13C10	3	6	1	0	30	3	3	0	0

* Infusion.

in the first tube within 10 to 15 minutes, with complete loss of motility in all tubes in 30 minutes. *Tetrahymena* appeared motile at the end of 20 minutes in the first tube, with no activity observed after 30 minutes. This protozoan remained active and motile in the ninth tube containing 0.2 ml of the extract at the end of 40 minutes, but no motility was observed in that tube or the tenth tube at the end of 60 minutes. The first tube contained 50 per cent solution of the extract and so on to the tenth tube which contained 9 per cent of the extract.

Toxicity tests. The amounts of crude saline and refined extracts that chickens and mice are able to tolerate are summarized in table 5. Observations were made at 10 and 60 minutes and at 24 hours. No attempts were made to determine the cumulative effects of the extracts as this could be observed in the *in vivo* experiments. The acute toxic symptoms helped to determine the levels which could be used in the *in vivo* tests.

The extracts, saline concentrate P24C10a and P24B1 of the waterleaf plant, and P13B31 extract of sagebrush were found to anesthetize animals receiving them. Waterleaf extract P24B1 produced (0.5 ml, i.p.) complete anesthesia for 4 to 5 hours when injected into mice. This same extract when given intravenously (0.5 ml) to chicks produced anesthesia and appeared to produce no toxic symptoms. P13B31 extract of sagebrush in a 1 per cent solution was toxic for mice if given intraperitoneally in 1-ml amounts. The animal went into a deep coma within a minute after injection and died within 7 minutes. The mice receiving 0.5 ml of P13B31, 1 per cent solution, were completely anesthetized in 5

TABLE 5

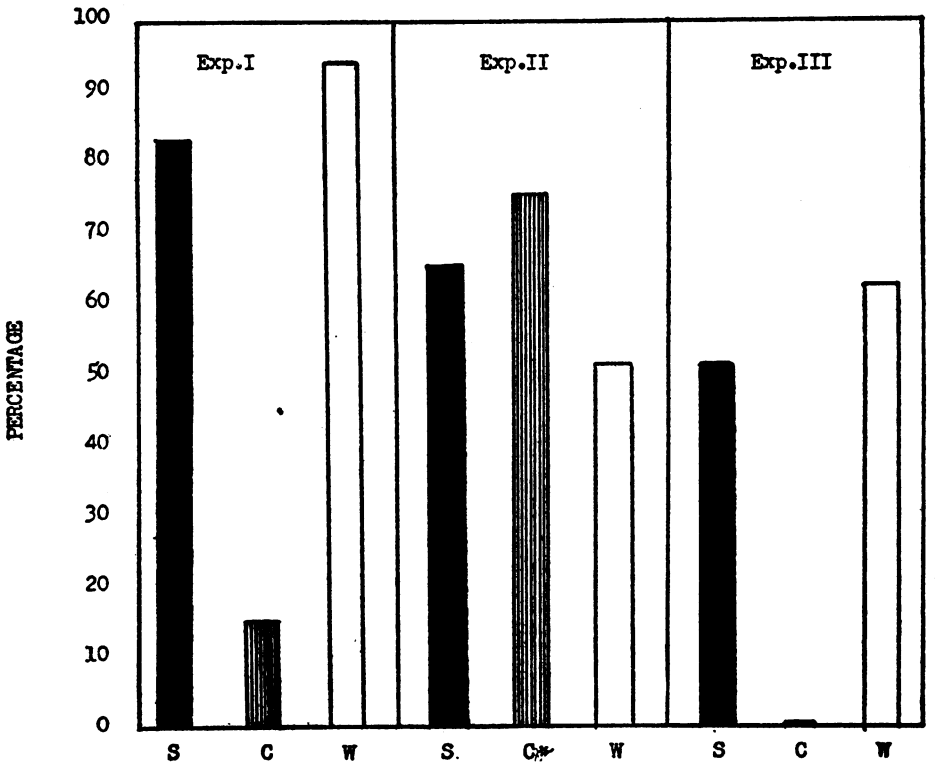
Toxicity of extracts of plants in chickens and mice

PLANT EXTRACTS	CHICKENS						MICE				REMARKS
	Intraperitoneal		Subcutaneous		Intravenous		Intraperitoneal			Subcutaneous	
	0.5 ml	1.0 ml	0.5 ml	1.0 ml	0.5 ml	1.0 ml	0.25	0.5 ml	1.0 ml	0.5 ml	
Buttercup											
P16C10	A	A	A	A			A	A	A	A	
P16B69			A	A							
P16B90			A	A			A	A	A	A	
Sagebrush											
P13C10			A	A			A	A	A	A	
P13B31—1%	A	D	A	D	A	D	A	A	D	D	Coma 0.5 i.p.
P13D24—1%	D	D	A	D			A	A	D	A	
P13C10a			A				D	A	A	A	0.25 i.p. Trauma
P13B31 1:400			A	A			A	A	A	A	0.5–1.0 i.p. Stagger
Waterleaf											
P24C10a	D	D	A	D			A	D	D	A	Partial paralysis before death i.p.
P24B1					A	A	A	A	A	A	Coma 0.5–1.0 i.p.
Mountain pasque											
P158C10	A	D	A	A			A	D	D	A	Subcutaneous blisters, chicks
P158B1a							D	D	D	A	
P158B1b							D	A	A	A	0.25 Trauma
P158B3			A	A			A	A	A	A	Subcutaneous blister
Juniper											
P14C10	A	D	A	A			D	A	A	A	0.25 Trauma
P14B1							A	A	A	A	
P14B2							A	A	A	A	
P14C10b			A				A	A	A	A	

A, alive after 24 hours.
D, died.

minutes. This stage lasted 5 to 10 minutes, at which time the animals began to revive. Further pharmacological and physiological studies are being undertaken on these two substances.

In vivo tests. A reduction in the total blood parasite counts of the treated groups as compared to the untreated control chickens was observed in the screen tests using the crude saline extracts. The original purpose of this study was to investigate substances separated from plants which were active against the blood stages. Therefore, if the infected chickens showed a definite and dramatic



GRAPH 1. PERCENTAGE OF CHICKENS DYING WITH EXOERYTHROCYTIC FORMS AFTER TREATMENT WITH SAGEBRUSH EXTRACT P13C10 AND WATERLEAF EXTRACT P24C10

S, sagebrush; C, controls; W, waterleaf. * —Quinine-treated controls.

decrease in blood parasites and longer survival time during treatment, the plant extract was considered to have potential possibilities even though the chicken later died of exoerythrocytic forms of the disease.

Sagebrush extracts P13C10 and P13C10a and waterleaf extracts P24C10 and P24C10a were tried using larger groups of chickens. In this experiment a total of 87 chickens were used. In experiment I (graph 1) using saline extracts of the two plants, 10 out of 12 chickens treated with sagebrush and 11 out of 12 chickens of the waterleaf-treated group died, with exoerythrocytic forms observed in

brain smears. Only 1 of the 6 chickens used in the control group was found to contain exoerythrocytic forms. These forms were observed as early as the tenth day of infection, with several birds of those specific test groups remaining completely free of the blood phase as determined by blood smears. The average day of death for chickens showing the presence of exoerythrocytic forms was 11.9 for those receiving sagebrush P13C10 and 11.7 with those receiving waterleaf P24C10. The one chicken in the control group succumbing to this form of the disease died on the sixteenth day.

Experiment I indicated that sagebrush P13C10 and waterleaf P24C10 extracts contained substances which were antimalarial in character in that less than 10 per cent of the waterleaf-treated and 20 per cent of the sagebrush-treated chickens died of the blood phase of malaria. The exoerythrocytic phase of the disease also appeared to be affected by the action of these two plant extracts. The presence of these forms was observed as early as the tenth day after inoculation, whereas the only chicken succumbing to the exoerythrocytic form in the control group died on the sixteenth day. With these findings in mind, a second experiment was done. To insure against the possibility of inoculating exoerythrocytic forms, donors with very early infections were used. The control group of chickens were to be treated with quinine HCl to ascertain if the plant extracts were enhancing the development of the exoerythrocytic forms of this infection. In experiment II, 9 of the 12 quinine-treated control group were observed with exoerythrocytic forms. All the birds succumbing to this form of the disease died between the seventeenth and nineteenth day of infection. Two chickens in the sagebrush group died on the eleventh day, and 5 on the sixteenth to the nineteenth day, with exoerythrocytic forms being observed in all brain smears. Six of the 12 chickens treated with waterleaf extract died, showing the presence of exoerythrocytic forms. The earliest death of this group with these forms did not occur until the nineteenth day.

Saline extract concentrates of the two plants, waterleaf P24C10a and sagebrush P13C10a, were used in experiment III (graph 1). With sagebrush, 4 out of 9 chickens died with exoerythrocytic forms, and those treated with waterleaf showed these forms in 5 of the 9 chickens. No exoerythrocytic forms were observed in the control group.

The survival time of the chickens treated with sagebrush extract in this group was found to be between 18 to 24 days, with one chicken remaining alive after becoming blood-parasite-negative; with waterleaf extract it was found to be between 13 to 24 days, the majority dying after the eighteenth day of infection; whereas in the control group all chickens had succumbed to blood infections between 8 to 11 days.

Further evidence of the antimalarial effect of sagebrush P13C10 and waterleaf P24C10 was shown by the fact that the parasite counts remained negative or less than 1 per cent throughout the infection, with death being due to exoerythrocytic forms. The blood smears were positive in the control chickens 4 to 5 days before they were positive in the test groups. The antimalarial effect was also observed in chickens with 50 to 70 per cent parasitemia. In these, continued treatment reduced the parasite count.

Extract P13B24 of sagebrush when tested as an antimalarial agent appeared to be active against the blood phase by reducing the parasite count in the screen test group, whereas extract P13B31 had no effect on the course of the infection in chickens. The latter extract, when given intraperitoneally, did cause the chickens to become stuporous. The antimalarial activity of P13B24 was further tested on a larger series of infected chickens. Three groups of 16 each were used. The first group received the drug 2 days before infection, the second group was treated as the chickens became positive, and the third group was the untreated group used as a control. In the pretreated group (I), 2 chickens died before becoming blood-positive and 4 died showing the presence of exoerythrocytic forms. The parasite counts of 5 chickens in this group remained less than 1 per cent or were reduced, 4 of these dying with exoerythrocytic forms. In group II, treated as they became positive, the results were very similar to those found in the chickens of the control group with the exception that 1 chicken died on the fourteenth day showing the presence of exoerythrocytic forms. All chickens in the control group had succumbed to the blood phase of the disease by the thirteenth day of infection.

Saline extract P14C10 of juniper, P158C10 of mountain pasque, and P16C10 of buttercup used as therapeutic agents against *P. gallinaceum* were found to contain no antimalarial activity when tested on larger groups of chickens. Refined extracts P158B1 and P158B3 of mountain pasque and P16B69 and P16B90 of buttercup were also found to have no apparent effect on chicken malaria.

Bacterial. The antibacterial effect *in vivo* of the saline and refined extracts was tested against *Diplococcus pneumoniae.* White Swiss mice were inoculated intraperitoneally with a 6-hour broth culture of type 19 pneumococcus. One hour after inoculation mice were injected with 0.5 ml of the extracts intraperitoneally and twice daily thereafter. Those animals alive after 24 hours were given further treatment. Five mice were used in the screening tests.

Of all the extracts tried (table 1) only one showed any action against the pneumococcus infection. The P158B3 extract of mountain pasque protected all mice at the end of 24 hours. All control animals were dead in the same period of time. Two mice of this screening group died in 48 hours with the other 3 remaining alive. Type 19 pneumococcus was isolated from the 2 mice dying in the treated group and from the control animals. To ascertain the *in vivo* activity of this drug in a larger group of animals, 50 mice were infected with type 19 pneumococcus from a 12-hour culture. The mice were divided into two groups of 25 mice each. One group was treated 1 hour after inoculation and twice daily thereafter. The second group was used for control and received no treatment. Six hours after inoculation all mice in the control group had succumbed to the pneumococcus infection. In the treated group all mice were alive at 6 hours, with 5 deaths occurring at the end of 8 hours. The remainder of the animals remained alive.

DISCUSSION

The foregoing experiments show that plants have distinct inhibitory substances which can be extracted and purified. Extracts of two plants, buttercup

(*Ranunculus occidentalis*) and mountain pasque (*Anemone occidentalis*), when tested for inhibitory substances by the petri plate method appeared to have very good diffusion. In some tests it was observed to have diffused throughout the seeded agar causing complete inhibition of growth of all organisms inoculated into the medium. Seegal and Holden (1945) have reported similar findings with buttercup, demonstrating definite inhibitory activity on a large series of microorganisms. As these plants, buttercup and mountain pasque, appeared far superior to any others tested, it was felt that they should be examined for a vapor which might prove to be soluble and have inhibitory characteristics. Such vapors were found. Fresh plants were always used in preparing all extracts. Buttercup and mountain pasque are closely related species in that they are of the same family, *Ranunculaceae*.

The P13C10 and P13B24 extracts of sagebrush and the P24C10 extract of waterleaf protected 50 per cent or more chickens during the blood phase of *P. gallinaceum* malaria. No apparent effect was observed on the exoerythrocytic forms. It was noted that the crude saline extracts (P13C10 and P24C10) protected larger numbers of infected chickens than the refined extracts of the plants.

The anesthetic activity of P13B31 extract of sagebrush and the P24B1 and P24C10a extracts of waterleaf was encountered when these extracts were being tested for toxicity. A more detailed report will follow at a later date on the pharmacological and toxicological aspects of these drugs.

It was felt by the authors that the plants which were observed to show activity against the blood phase of the malaria parasite would most likely be active against bacterial infections. Our results did not bear out this hypothesis. The P158B3 extract of mountain pasque was the only plant extract which protected animals against bacterial infections.

No attempt has been made at this writing to ascertain the specific chemical activity of any of these extracts.

SUMMARY

The saline extracts of five plants (buttercup, sagebrush, dwarf waterleaf, mountain pasque, and juniper) out of more than 200 tested were found to have antibacterial and antimalarial activity by *in vitro* methods.

One plant, buttercup, was found to inhibit the growth of many gram-negative and gram-positive bacteria, fungi, soil organisms, and nonpathogenic protozoa by *in vitro* methods.

Mountain pasque and buttercup were found to contain vapors which were bacteriostatic and bactericidal. Volatile oils separated from the same plants were found to exhibit similar activities.

An extract of mountain pasque was found to protect mice heavily infected with the pneumococcus.

Two plants, sagebrush and dwarf waterleaf, contained substances that protected chickens during the blood phase of malaria.

An incidental observation revealed that the same two plants possessed anesthetic properties in chickens and mice.

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