

preparations of juice and two distillates examined gave similar results. However, the measurements of inhibition of growth varied somewhat with different preparations and with the same preparation tested on different days. The figures given in the table represent the minimum and maximum inhibition observed.

H. streptococcus, 15A, was tested for its ability to grow in blood broth in the presence of buttercup juice. Quantities of buttercup representing 0.5 cc, 0.25 cc, 0.12 cc and 0.05 cc of undiluted juice were added to 6 or 7 cc of blood broth which together with controls were seeded with 0.5 cc of a 10^{-3} dilution of a blood broth culture of the streptococcus. The pH of the media was 7.7 before incubation, while after 24 hours' incubation it varied from pH 6.7 to 7.5. After incubation the number of viable organisms were determined by pouring serial dilutions of the cultures. In two experiments the 0.5 cc of buttercup juice inhibited all growth. In a third experiment with another preparation of juice growth was decreased to 10 per cent. of that occurring in the control tubes. Twenty-five hundredths and 0.12 cc of buttercup juice depressed growth also. Five hundredths cc was without effect on the number of organisms growing in 24 hours.

The effect of buttercup juice on the growth of *My. tuberculosis hominis* was tested by adding 5 cc, 2.5 cc or 1.25 cc of undiluted juice to 100 cc quantities of Sauton's media, which was then seeded with H37RV.⁸ Growth failed to occur during a month of observation in any of the nine flasks. The three control flasks showed the usual growth.

The effect of buttercup juice and distillate were tested on the growth of *Candida* (*Monilia*) *albicans*, *Candida* (*Monilia*) *krusei* and *Cryptococcus hominis*.⁹ Pour plates were prepared from glucose agar melted and cooled to 50° C. to which 0.5 cc, 0.25 cc, 0.12 cc or 0.05 cc of juice was added; all tubes together with untreated controls were seeded with the three microorganisms. The plates were observed at room temperature for two weeks. Five one-hundredths of a cc of juice sufficed to prevent all growth of *Candida krusei* and *Cryptococcus hominis*. Twenty-five hundredths of a cc prevented all growth of *Candida albicans*. All control plates had profuse growth of colonies too numerous to count. In the case of *Candida albicans* the steam distillate obtained from the pressed juice was also tested. One-half cc of this added to the agar resulted in complete inhibition of growth.

Dried plants (*A. pulsatilla*) were obtained from S. B. Penick Co., through the courtesy of Dr. Hock-

⁸ We are indebted to Dr. M. M. Steinbach for preparing these cultures.

⁹ We are indebted to Dr. Rhoda Benham for these cultures.

ing. These were ground, weighed and four times the weight of water added. A preparation of juice and a distillate were obtained as in the case of buttercup. Both were tested against *H. streptococcus* 15A, by the Oxford cup method. The radius of the zone of inhibition was from 1.0 to 1.5 cm. The distillate also was tested against *Candida albicans* by adding 0.5 cc to pour plates of the organism. No growth of the *Candida albicans* occurred. The antibiotic activity of this plant thus compared favorably with that of buttercup.

The toxicity of the buttercup juice for laboratory animals has prevented its use in the therapy of infection. The distillate is less toxic to animals than is the whole juice. Chemical methods are being developed in an attempt to separate the toxic from the antibiotic substance.

CONCLUSION

Pressed juice or steam distillate from the pressed juice of buttercup (*Ranunculaceae* family) is a strong antibiotic with a wide range of activity. It has proven effective *in vitro* in inhibiting the growth of selected Gram-positive and Gram-negative pathogenic cocci and bacilli, *Mycobacterium tuberculosis* and three yeasts, two of which are potential human pathogens.

BEATRICE CARRIER SEEGAL
MARGARET HOLDEN

DISTRIBUTION OF RADIOACTIVE SULFUR IN THE RAT

In the course of an investigation of the effects of carbonyl bisulfite on rats bearing tumors, it became desirable to study the distribution, in the animal, of the sulfur-containing moiety of these molecules. The intraperitoneal injection of two carbonyl bisulfites and of sodium sulfate, all containing radioactive sulfur (S^{35}), led to an unexpected accumulation of the active material in the bone marrow of the animal.

Heptylaldehyde bisulfite and cinnamaldehyde bisulfite containing S^{35} were synthesized by passing $S^{35}O_2$ under nitrogen into a slightly alkaline solution containing the aldehydes. Sodium sulfate was synthesized by bubbling $S^{35}O_2$ into an excess of alkaline hydrogen peroxide under a nitrogen atmosphere. All the solutions were adjusted to pH 7.4 and then injected intraperitoneally into rats.

The animals were given water ad lib but were given no food for a period of 14 to 16 hours, at the end of which they were sacrificed. The various tissues were dissected out and aliquots removed for weight and radioactivity measurements. The tissue was decomposed by alkaline fusion,² the melt neutralized, and

¹ We are indebted to Dr. M. L. Crossley, of the American Cyanamid Company, for the radioactive sulfur.

² K. Bailey, *Biochem. Jour.*, 31: 1406-1413, 1937.

the sulfate precipitated as the barium salt. The precipitate was collected following the usual method.³

Measurements of radioactivity were made with a well-shielded G.M. counter tube provided with a thin mica window of the type described by Copp and Greenberg⁴ regulated with a Neber-Haper circuit. Counts were registered by a scale of eight circuit energized by a stable 115 volt A.C. line.

Typical results obtained with the three compounds mentioned above are shown in Table 1. All values are

TABLE 1
DISTRIBUTION OF S³⁵ IN RAT TISSUES

	Heptyl- aldehyde bisulfite	Cinnamal- dehyde bisulfite	Sodium sulfate
Total S ³⁵ injected, counts /min. $\times 10^{-3}$	8.5	110	118
Body weight, gms	115	165	175
Tissue	Counts	/ min.	/ 100 mgm
Bone marrow	218	280	208
Bone matrix	10	14	45
Lymph nodes	4	23	36
Spleen	4	11	18
Thymus	2	7	33
Lymphosarcoma*	4
Liver	3	18	10
Kidney	6	15	39
Brain	3	45	8
Hair	11	130	26

* We wish to thank Dr. J. B. Murphy, of the Rockefeller Institute for Medical Research, for the rats bearing lymphosarcoma tumors used in these experiments.

corrected for radioactive decay and for beta ray absorption in the samples.

It is evident from the table that the highest concentration of radioactive sulfur occurs in the bone marrow irrespective of the sulfur compound used. Though comparison between animals can not be readily made, the data show, nevertheless, that the relative distribution of sulfur in some tissues is somewhat dependent upon the chemical structure of the compound.

Preliminary chemical analysis of the marrow indicates an unexpectedly high sulfur content. The nature of the substances responsible for this high sulfur value are, at present, unknown.⁵

H. O. SINGHER
L. MARINELLI

MEMORIAL HOSPITAL,
NEW YORK CITY 21

EXPERIMENTAL VERRUCOUS ENDOCARDITIS¹

ON June 11, 1943, we received from Dr. Jacob Werne, of St. Johns Hospital, Long Island City, a

³ We wish to thank Dr. J. R. Rachele, of the Department of Biochemistry of Cornell Medical College, for assistance in setting up a method to collect the radioactive precipitates.

⁴ D. Harold Copp and David M. Greenberg, *Rev. Scientific Instruments*, 14: 205-206, July, 1943.

⁵ We wish to thank G. Horiuchi and H. Levy for technical assistance.

¹ Aided in part by the United Hospital Fund of New York; Grants No. 522 and No. 523 of the Committee on

specimen of fluid removed from the pericardial sac of a woman who had died of active rheumatic carditis. This fluid was passed through a Mandler filter and the filtrate was introduced into culture media (aerobic) with negative result. Some of this filtrate was injected intravenously into rabbits in which there were subsequently found scattered foci of inflammation in the myocardium, in one or more of the valves of the heart and in the pulmonary arterioles. The changes in the valves of the heart could be distinguished from those seen in experimental bacterial endocarditis because of the absence of bacteria, the more diffuse dissemination of the lesions, their curious edematous verrucous character and especially by the remarkable hyperplastic and reparative activity of the endothelium and connective tissue.

During the subsequent eighteen months the pericardial exudate from two more rheumatic patients and the blood from seven others with clinical evidence of rheumatic carditis have been injected into rabbits, guinea pigs and mice, with somewhat inconstant results. It has been possible to recognize damage of endocardial endothelium and connective tissue in most of these animals. The experimental disease has ordinarily not been lethal and the animals have been sacrificed at various intervals after inoculation.

The disease has been propagated in series through fifteen successive animals by injection of blood. In embryonated eggs, inoculation with small amounts of blood from the experimental mammals has given rise to a non-lethal infection which has been propagated in series in the eggs. Allantoic fluid of the eggs has been in turn injected intravenously into rabbits with the production of even more pronounced lesions in the heart, apparently because of a greater concentration of the pathogenic agent in the egg as compared with the mammalian blood.

In addition to the many rabbits examined in our studies of experimental bacterial endocarditis there have been animals injected with normal rabbit blood, blood of animals dead of various spontaneous disorders, animals injected with normal human blood, normal egg fluids, vaccinia virus, Theiler virus, influenza virus and with the blood of rabbits in which there was evidence of spontaneous disease of the heart. The changes produced in these animals require much further study. At present, however, it appears that they are not identical with the changes seen in the animals of the "rheumatic" series. The distinction between these various disorders of the rabbit's

Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association; and by the Virus Research Fund of the Lambert Pharmacal Company. This paper was presented in part, together with demonstration of specimens, at the meeting of the New York Pathological Society, New York Academy of Medicine, March 22, 1945.