an elongation of the refractory period and no hint that some of the cells may be stimulated subliminally, we may assume that the decrease in the potential size

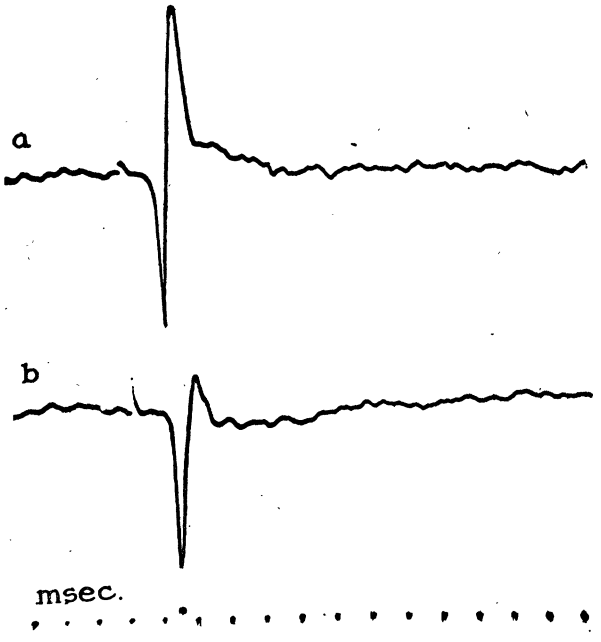

FIG. 1. Potential of ventral horn evoked by stimulation of tibial nerve and recorded by micro-electrode in 7th lumbar segment of the spinal cord of a cat. *a.* Normal side. *b.* Side altered by 27 days retrograde degeneration.

is due to a decrease in the external potential fields of the component cells of the nuclei. That this is apparently the equivalent of the loss of the ability to transmit the proprioceptive reflex offers a new field for theoretical consideration of interneuronal transmission.

CONCLUSION

Cells which have undergone chromatolysis as a consequence of peripheral nerve section respond to antidromic stimulation with reduced action potentials. No indication that any of the cells are stimulated subliminally is found in conditioning curves and the conclusion is drawn that the evoked external potential field of the individual cells is decreased in amplitude.

BERRY CAMPBELL

THE MEDICAL SCHOOL,
UNIVERSITY OF MINNESOTA,
MINNEAPOLIS

THE ANTIBIOTIC ACTIVITY OF EXTRACTS OF RANUNCULACEAE[1]

SINCE the observation of Boas[2] that the leaves and stems of some species of Ranunculaceae contain a soluble substance which retards the growth of *Rhizopus nigricans,* several reports on the antibiotic properties of this family of plants have appeared.[3, 4, 5, 6] Extracts from two members of the Ranunculaceae (buttercups and *A. pulsatilla*) have been examined for their antibiotic activity in this laboratory.[7] Two types of extract were prepared as follows from buttercup, the plant more extensively investigated. The first, referred to as juice, was obtained by grinding the stems, leaves and blossoms of freshly picked buttercups in a meat-chopper, adding an equal weight of distilled water, mixing thoroughly and pressing out the juice through gauze. This was then autoclaved at 15 pounds pressure for 20 minutes. The fluid so obtained was greenish-brown, opalescent and of approximately pH 6.5. The second type of extract, referred to as distillate, was obtained from the juice by steam distillation. The distillate was water clear and of approximately pH 7.1.

The two types of buttercup extracts have been tested for their antibiotic activity against a number of cocci and bacilli which are listed in Table 1. The method of testing consisted in streaking blood agar plates with broth cultures of the organisms and adding Oxford cups filled with the test substance. After 24 hours' incubation at 37° C. the size of the zone of inhibition was measured. It will be seen from the table that all the organisms were sensitive to the antibiotic action of buttercup extract. The thirteen

TABLE 1

THE ANTIBIOTIC ACTIVITY OF BUTTERCUP JUICE AND DISTILLATE TESTED BY THE OXFORD CUP METHOD ON A SELECTED GROUP OF BACTERIA

Bacterium	Radius of inhibition of growth	
	Juice cm*	Distillate cm*
H. strep. 15A	0.9–3.0	1.5–3.0
H. strep. C203	1.5–2.2	1.1
Strep. viridans	0.9	0.5–1.5
Pneumococcus I	1.6	1.5
Pneumococcus III	0.6–1.0	0.6–1.0
N. catarrhalis	1.8	3.0
Staph. aureus BD	0.6	1.0
Staph. aureus Pos.	0.4	0.3–0.6
Staph. Oxford	0.5	1.0
B. anthracis	0.6	0.3–0.6
Pseudomonas aeruginosa (*B. pyocyaneus*)	0.6	0.5–0.7
S. schottmülleri (*B. paratyphosus* B)	0.5	0.6
Esch. coli	0.5	0.5

* The figures given represent the minimum and maximum radius of inhibition observed. When penicillin was employed as a control utilizing *H. streptococcus* 15A, C203 and *Staph.* Oxford as test organisms a solution reported to contain 3 units per cc produced a zone of inhibition with a radius of 0.8–1.0, 1.1 and 0.9–1.0 cm, respectively.

[1] From the department of bacteriology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

[2] Friedrich Boas, *Ber. deut. botan. Ges.,* 52, 126, 1934.

[3] Friedrich Boas and Rudolph Steude, *Biochem. Z.,* 279, 417, 1935.

[4] Friedrich Keding, *Angew. Botan.,* 21, 1, 1939.

[5] Gisela Schmidt, *Z. f. Imm.,* 102, 233, 1942.

[6] E. M. Osborn, *Brit. Jour. Exp. Path.,* 24, 227, 1943.

[7] We are indebted to Miss Gertrude Herz for technical assistance.

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. Hocking. 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

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

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

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}[1] 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.