Rh_0 factor. In this way reactions could occur due to residual anti-Rh_0 agglutinins in the serum, which might erroneously be attributed to the anti-Rh′ or anti-Rh″ agglutinin. This pitfall can now be eliminated with the aid of potent anti-Rh_0 blocking serum,[18] because if such a serum is mixed with a diluted anti-Rh_0′ or anti-Rh_0″ serum, the action of the anti-Rh_0 agglutinin will be completely eliminated. An alternative technique is to retest the blood sample with the anti-Rh′ and anti-Rh″ sera after the blood has been treated with anti-Rh_0 blocking serum in order to block the Rh_0 antigen.

The author feels that these observations concerning the intermediate genes probably complete the picture of the Rh blood types. Should, however, other Rh antisera be encountered with specificities different from those of the known antisera, it should not be too difficult to adjust the nomenclature in order to include them in the scheme. For example, if a new agglutinin anti-Rh‴ is discovered, analogous to anti-Rh′ and anti-Rh″, it would only be necessary to postulate the existence of two additional genes Rh_3 (or Rh_0‴) and Rh‴, and the scheme of 8 Rh types would be enlarged to a scheme of 14 types as shown in Table 5.

TABLE 5

SCHEME OF THE RH TYPES EXTENDED TO INCLUDE A HYPOTHETICAL AGGLUTININ ANTI-RH‴

Blood lacking Rh_0					Blood containing Rh_0				
	Reactions with antisera					Reactions with antisera			
Types	Anti-Rh′	Anti-Rh″	Anti-Rh‴	Anti-Rh_0	Types	Anti-Rh′	Anti-Rh″	Anti-Rh‴	Anti-Rh_0
Rh-neg.	−	−	−	−	Rh_0	−	−	−	+
Rh′	+	−	−	−	Rh_1(Rh_0′)	+	−	−	+
Rh″	−	+	−	−	Rh_2(Rh_0″)	−	+	−	+
Rh‴	−	−	+	−	Rh_3(Rh_0‴)	−	−	+	+
Rh′Rh″	+	+	−	−	Rh_1Rh_2	+	+	−	+
Rh′Rh‴	+	−	+	−	Rh_1Rh_3	+	−	+	+
Rh″Rh‴	−	+	+	−	Rh_2Rh_3	−	+	+	+

As has been pointed out elsewhere,[19] the anti-Hr sera have a place in the scheme of the Rh blood types similar to that of the anti-O sera in the blood group scheme. The newer knowledge of the Hr factor[20] has therefore helped to clarify the problem of the nature of the anti-O sera. If, as seems probable, anti-O sera react with the properties determined by genes O and A_2, but not with those determined by genes A_1 and B, then it is clear that only bloods of genotypes A_1B, A_1A_1 and BB will fail to react with potent anti-O sera (*cf.* Table 6). The confusion that existed up to now

[18] A. S. Wiener, *Proc. Soc. Exp. Biol. and Med.*, 56: 173, 1944.

[19] A. S. Wiener and H. Karowe, *Jour. Immunol.*, 49: 51, 1944.

[20] A. S. Wiener, I. Davidsohn and E. L. Potter, *Jour. Exp. Med.*, in press.

TABLE 6

REACTIONS OF ANTI-O SERA WITH BLOODS OF THE VARIOUS GROUPS

Blood of group	Reactions with antisera				Reaction with anti-O serum
	anti-A	anti-A_1	anti-B	Genotype	
O	Neg.	Neg.	Neg.	*OO*	Strong
A_1	Pos.	Pos.	Neg.	*A_1A_1*	Neg.
				A_1A_2	Weak
				A_1O	
A_2	Pos.	Neg.	Neg.	*A_2A_2*	Strong
				A_2O	
B	Neg.	Neg.	Pos.	*BB*	Neg.
				BO	Weak
A_1B	Pos.	Pos.	Pos.	*A_1B*	Neg.
A_2B	Pos.	Neg.	Pos.	*A_2B*	Weak

was caused by the fact that anti-O sera, like anti-Hr sera, are usually of low potency, so that consistent positive reactions were obtained only with bloods of groups O and A_2. Wtih more potent anti-O sera, the reactions obtained agree closely with the predictions under the theory proposed above.

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ANTIBACTERIAL SUBSTANCES IN ORGANS OF HIGHER PLANTS

THE presence in higher plants of substances with antibacterial properties has been reported in previous publications.[1, 2, 3, 4, 5, 6, 7] The work of Fleming, Florey, Waksman and many others has shown that such substances present in lower plants have important biological significance. Hence, a more intensive effort in investigating the vast field offered by higher plants seemed to be warranted. The authors have for the past six months been engaged in a systematic review of members of families of higher plants with the aim of discovering and isolating substances with antibacterial properties. Osborn's[8] work in the same field was not known to the authors when this search was begun, hence some duplications occurred.

The hypothetical foundation of this search was twofold. (1) It was known that in the rizosphere of higher plants the growing roots regularly survive the actions of innumerable micro-organisms capable of destroying them. It was concluded that a mechanism must be present in the plants enabling them to counteract potentially destructive microorganisms. One such mechanism is undoubtedly manifested in the secretion of acids within the rizosphere. Other protective mechanisms in plants might be based on antibiotic substances of a specific nature. (2) For centuries plant drugs have been used in all parts of the

[1] E. Glaser and F. Prinz, *Fermentforsch.*, 9: 64, 1926.
[2] O. Stickl, *Zeits. Hyg. Infektkr.*, 108: 566, 1928.
[3] F. Boas, *Ber. D. Bot. Ges.*, 52: 126, 1934.
[4] F. Boas and R. Steude, *Biochem. Zeits.*, 279: 417, 1935.
[5] F. Boas, *Ber. D. Bot. Ges.*, 57: (100), 1939.
[6] O. E. Böcker, *Zeits. Hyg. Infektkr.*, 121: 166, 1939.
[7] Other references are given in the paper of Huddleson *et al.*, published in *Jour. Vet. Asn.*, 105: 394, 1944.
[8] E. M. Osborn, *Brit. Jour. Exp. Path.*, 24: 227, 1943.

world as folk remedies. While some drugs have been accepted for medicinal purposes, most of them have still remained in disregard and their employment has often been relegated into the field of superstition. It was thought that some of these plants might contain principles adverse to the development of undesirable microorganisms in the human body.

The search was, therefore, undertaken with particular consideration (1) of the root system of plants, and (2) of plants mentioned in the folklore of numerous countries as beneficial in their application in cases of bacterial infections.

EXPERIMENTAL

Method. The activity of plant extracts and of their concentrates was tested by a method similar to the one described by Sherwood *et al.*,[9] which is a modifi- by British workers[10] in connection with penicillin research. Sterile filter paper discs (Schleicher and Schuell No. 470) of 12.8 mm diameter were used as carriers of the solutions to be tested. The results were evaluated in a qualitative way by arranging them in groups according to the diameters of zones of inhibition.

Procedure. The plant samples were extracted in a Waring Blendor with four times their weight of distilled water. The extracts were strained through cotton cloth, filter paper discs were dipped into the filtrate and placed on the agar of prepared Petri dishes. In most instances, four test organisms were used: *Staphylococcus aureus, Escherichia coli, Phytomonas phaseoli* and *Phytomonas campestris.* The first two organisms were incubated at 37° for 18 hours; the two plant pathogenes grew at room temperature for 48 hours. After these periods any zones of inhibition were measured.

RESULTS

The search of which this report gives a preliminary account has shown that some higher plants contain antibacterial principles at certain stages of their development. These principles vary greatly as to their potency and distribution within the plants. However, regularity of occurrence under identical conditions was established in most of the plants studied. Thus, the leaves of *Onopordon Acanthium,* the Scotch thistle, contain a principle inhibiting the growth of *Staphylococcus aureus.* However, it is only the second year's growth that produces an active extract in appreciable strength. No other thistles examined contained this principle. Other plants exhibiting similar activity in leafy tissues are *Verbascum Thapsus* and *Paeonia officinalis.*

The authors are in accord with Osborn that certain plants tested by others using different procedures do not give a positive response in the plate test. Horseradish, turnips and *Chelidonium majus* were negative in tests made, as were several cabbage varieties. It is believed that the failure of these and of some other plant extracts to respond positively to the plate test is due to the low degree of their diffusibility.

Fruits of certain representatives of the genera *Lonicera* (Fam. *Caprifoliaceae*), *Vaccinium* (Fam. *Ericaceae*), *Ribes* (Fam. *Saxifragaceae*) and *Sorbus* (Fam. *Rosaceae*) are the carriers of active substances.

Some varieties of *Lonicera tatarica,* one of the honeysuckles, have been studied rather extensively, and a potent substance has been discovered which inhibits the growth of *Staphylococcus aureus* and *Escherichia coli.* The inhibition of the gram-positive organism is more pronounced than that of the gram-negative (Principle A in Table 1). In addition, the fruits contain another principle, not active against gram-positive organisms but strongly interfering with the growth of *E. coli* (Principle B in Table 1). Both principles are extractable with water, ethanol and methanol. Principle B can also be extracted with dioxan. Both principles are less soluble in ethyl acetate, nearly insoluble in chloroform and insoluble in ethyl ether, petroleum ether, toluene and carbon tetrachloride. They are heat stable in boiling water for some time; however, boiling for several hours destroys them.

TABLE 1

ACTION OF PLANT EXTRACTS TOWARD BACTERIA. (CONCENTRATIONS ARE COMPARABLE. THE MOST SUITABLE SOLVENT WAS USED IN EACH CASE)

	Paeonia officinalis	*Onopordon Acanthium*	*Vaccinium corymbosum*	*Lonicera tatarica* Principle A	*Lonicera tatarica* Principle B	*Sorbus Americana*
Staph. aureus ..	+	+	..	++	..	+++
E. coli	+	..	++++p	+	++++p	++++
Ph. phaseoli ...	not tested		+++	++++	++++p	++++
Ph. campestris .	not tested		++	++	++++p	+++

Ranges of inhibition: + = < 20 mm diameter; ++ = 20–25 mm diameter; +++ = 26–30 mm diameter; ++++ = > 30 mm diameter; p = partial inhibition.

Some of Osborn's general observations could be confirmed, for example, the similarity of antibacterial actions throughout a genus. However, considerable differences in the potency of the active principles were noted within genera and even species. The active principles found in the scarlet berries of *Lonicera tatarica* were not present in species and varieties with dark red, orange, yellow and purple fruits.

[9] M. B. Sherwood, E. Falco and E. J. DeBeer, SCIENCE, 99: 247, 1944.
cation of the plate assay method recently described

[10] E. P. Abraham, E. Chain, E. M. Fletcher, H. W. Florey, A. D. Gardner, N. G. Heatley and M. A. Jennings, *Lancet,* II: 177, 1941.

In addition to the inhibitory effect of some plant extracts a peculiar phenomenon of disturbed growth and very often of definite stimulation of the test organisms was observed. These phenomena were similar to those described by Abraham *et al.*[10] They appeared as halos of varying sizes surrounding the zones of inhibition. The stimulation was in some cases of extraordinary strength. Boas[3] called attention to the fact that stimulative principles may be present in plant tissues together with inhibitors. A simultaneous action in this sense might explain the observation mentioned. It is also regarded possible that the inhibitor as it penetrates the agar becomes diluted to such a degree that its action reverses.

The results obtained so far indicate that a wide field is opening up for exploration. In all probability, the problems to be encountered will be of a general biological nature rather than being confined to the interrelations between bacteria and higher plants. Broader aspects are coming into the picture, heretofore merely touched but not yet developed.[1, 3, 5]

The authors envisage the applicability of some of the findings to plant pathological problems, particularly in connection with the treatment of seed-borne diseases not yet controllable. An indication of the effect of an antibiotic on plant pathogenes is given in the work of Brown and Boyle[11] in which two plant pathogenes, *Erwinia carnegieana* and *Corynebacterium sepedonicum,* are shown to be inhibited by penicillin. In the light of the work of Link and Walker[12] it is not illogical to suspect that the resistance of some plants to disease is due to the presence in the host cells of distinct chemical substances which are antibiotic to specific pathogenes. The development of antifungal principles, similar in their action to the antibacterial substances, is not outside the realm of possibility.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW PROCEDURE FOR THE BIOASSAY OF VITAMIN C[1]

THE basis of an acceptable bioassay procedure for the estimation of vitamin C in foods is a test diet which is devoid of vitamin C but otherwise adequate for the growth and development of the guinea pig. Commonly used diets amply fortified with ascorbic acid have been shown in this laboratory to be nutritionally incomplete for normal growth and reproduction of these animals.

As a result of studies over the past four years involving some 2,000 guinea pigs, a ration mixture has been devised which appears to give promise in vitamin C bioassays. It is vitamin C—free; is palatable enough to be readily eaten; supports normal reproduction of mature females and practically normal growth of the young to maturity and subsequent reproduction; and consists of ingredients of relatively low nutritional variability, thus permitting reproducible results in growth or tooth development. (Normal performance was taken as that obtained on a mixture similar to the one herein reported plus green feed fed ad libitum.)

The feed mixture consists of:

15.0	per cent.	Ground No. 1 feed oats
10.5	" "	Ground No. 5 Canadian northern wheat
25.0	" "	Dried beet pulp
10.0	" "	Linseed oilmeal
15.0	" "	Dried skimmilk
5.0	" "	Non-oily fish meal
5.0	" "	Wheat germ meal
10.0	" "	Dried brewers yeast
4.0	" "	Bone char
0.5	" "	Salt (iodized at 0.1 per cent. KI)

This mixture for feeding is pressed into pellets of about 1/16 inch diameter and 1/4 inch length. In addition to these pellets, guinea pigs are fed every second day by direct administration 0.5 cc of a feeding fish oil to supply vitamins A and D. On alternate days they are given 6 mg alpha tocopherol. These quantities are doubtless in excess of the unknown minimum requirements. Adequate ascorbic acid (not less than 2 mg/day for growing pigs) must of course also be supplied. The significance of the proportions of the diet mixture ingredients is unknown. Doubtless considerable change in the quantities of most of the foods may be made, and some may probably be omitted or replaced with others. The reduction of this formula to one consisting of purified materials is the object of studies now under way at this station.

Using this diet the eight-weeks growth response of young male guinea pigs to graded doses between 0.5 mg and 2.0 mg of ascorbic acid bears a linear relation to the log log of the dose. Female pigs have been found unsatisfactory in growth assays because of higher variability and slower growth rates. The variability in growth response in the male pigs on identical dose levels of the order of 29 per cent. and with 16 pigs per group it required 40 to 45 grams differ-

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 197.

[11] J. G. Brown and A. M. Boyle, *Phytopathology,* 43: 760, 1944.

[12] K. P. Link and J. C. Walker, *Jour. Biol. Chem.,* 100: 379, 1933.