and perhaps should have been classified as intermediate types. Intermediate types are frequent in Negroes.[7]

[7] Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 89.

The difference in incidence of the Rh types may well be explained on the basis of some unknown mixture with the colored race, which differs in regard to the incidence of the different Rh types from the white American population.

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Influence of Sulfonamides and Para-aminobenzoic Acid on the Growth of *Lupinus albus.*

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The mode of action of sulfanilamide is still an unsolved problem. Various hypotheses based more or less on experimental data have been advanced, as for instance the catalase hypothesis, the respiration-interference hypothesis, the oxidation hypothesis, and others, described by Long[1] and by Henry.[2] Perhaps the most widely accepted explanation is the so-called PABA theory. Since under certain conditions para-aminobenzoic acid (PABA) neutralizes the bacteriostatic effects of sulfanilamide, it has been suggested that the influence of PABA is a result of its competition with sulfanilamide for a place in certain enzyme systems of susceptible bacteria. In the use of seedlings and other botanical test objects for pharmacological studies[3,4] it seemed of interest to investigate the effects of para-aminobenzoic acid on the one hand and of sulfonamides on the other, separately and in combination with each other, on the growth of higher plants. The results have been partially reported[5] and are given more fully here.

Method. The biological criterion employed was the elongation or growth of the well-defined straight roots of Lupinus seedlings, studied by the author's phytopharmacological methods, details of which have been published.[6] The procedure was briefly as follows.

Seeds of *Lupinus albus,* large variety, were soaked in tap water overnight and planted in finely ground sphagnum moss containing adequate moisture. After germination, the growth of selected seedlings with roots 35 to 50 mm long was studied in a solution consisting of equal parts of distilled water and Shive physiological saline.[7] The various drugs, sulfonamides and PABA, were dissolved in the same hydroponic medium, *i.e.,* half distilled water and half Shive solution. At least 10 seedlings, placed in hard-glass test tubes, were employed for the control solution and for each drug solution, in the different concentrations used. The sharply defined straight roots were measured at the beginning and end of every experiment, and the ratio of growth of the seedling roots in the various drug solutions is expressed as a percentage of their growth in the control solution according to the formula,

$$\text{Index of Growth} = \frac{\mathrm{X}}{\mathrm{N}} \times 100$$

in which N represents average growth of controls and X the average growth in the drug solution. All of the seedlings were allowed to grow for 24 hours in the dark at 15°C.

Substances Studied. The investigation may

[1] Long, P. H., *Sigma Xi Quarterly,* 1941, **29**, 149.

[2] Henry, R. J., *Bact. Rev.,* 1943, **7**, 175.

[3] Macht, D. I., *J. Pharm. and Exp. Ther.,* 1926, **29**, 461.

[4] Macht, D. I., *Science,* 1930, **71**, 302.

[5] Macht, D. I., and Kehoe, D. B., *Fed. Proc.,* 1943, **2**, 30.

[6] Macht, D. I., and Macht, M. B., *J. Lab. and Clin. Med.,* 1941, **26**, 597.

[7] Shive, J. W., *Physiol. Rev.,* 1915, **1**, 327.

be divided into three parts: (1) The influence of benzoic acid and of its ortho-, meta-, and para-amino derivatives; (2) the effect of a number of sulfonamides in various concentrations, including sulfanilamide, sulfathiazole, sulfapyridine, sulfadiazine, sulfaguanidine, succinyl sulfathiazole, and neoprontosil; (3) a more extensive study of sulfanilamide in various concentrations, of PABA in various concentrations, and of mixtures of the two, to ascertain their effect on each other.

Results. Effect of Benzoic and Aminobenzoic Acids. Benzoic acid and sodium benzoate have been previously studied by Macht and Livingston, and were found to be exceedingly toxic for plants.[8]

Twenty experiments were performed on the comparative toxicity for the *Lupinus albus* seedlings of the 3 amino-benzoic acids. The pH of the various concentrations employed in Shive's solution varied but little (pH 4.8-pH 5.2) and it has been previously determined that even much wider differences in the pH of Shive solutions had little effect on the growth of lupines. Of the 3 amino-benzoic acids the meta variety was the most toxic, and the para variety the least toxic. For illustration, the growth index of Lupinus seedlings in concentrations of 1:20,000 dissolved in Shive solution was 31 for *m*-benzoic acid, 50 for *o*-benzoic acid, and 68 for PABA.

It will be shown later that PABA, while inhibiting growth in the above concentration (1:20,000), actually stimulates growth in highly dilute solutions.

Effects of Various Sulfonamides. Of the 7 sulfa compounds studied for comparative purposes 20 experiments were made with each. Growth in fairly high concentration (1:10,000) revealed that sulfanilamide produced the greatest inhibition (Table I). Sulfathiazole, sulfapyridine, and sulfadiazine were less inhibitory in 1:10,000 while sulfaguanidine, succinyl sulfathiazole, and neoprontosil were actually stimulating to root growth in that concentration.

This stimulating effect of the last 3 compounds prompted further study of the first 4, namely, sulfanilamide, sulfathiazole, sulfapyridine, and sulfadiazine in weaker dilutions. It will be seen (Table I) that those 4 sulfonamides which produced some inhibition of growth in concentration of 1:10,000, also produced stimulation at lower concentrations. This biphasic or diphasic action of drugs on plant growth is not an unusual phenomenon in plant physiology and pharmacology. Thus, the 3 auxins or plant hormones, indole-acetic, indole-butyric, and naphthalene acetic, acids while stimulating root growth in very weak doses, actually inhibit growth when used in greater concentrations.[9] The statistical reliability of the figures obtained with 10 or more plants in any one experiment has been fully discussed elsewhere[6] and the same degree of validity applies to the data of the present research. As regards the comparative data obtained in different experiments performed with different crops of plants on different days the variations in the results did not vary more than 5 to 10%.

TABLE I.
Effect of Higher Dilutions of Aqueous Solutions of Various Sulfa-Compounds on Growth of Lupinus Seedlings.

Drug	Concentration × 10,000	Phytotoxic index
Sulfanilamide	1:1	68
"	1:10	86
"	1:25	112
Sulfathiazole	1:1	86
"	1:10	90
"	1:25	118
Sulfapyridine	1:1	88
"	1:10	94
"	1:25	130
Sulfadiazine	1:1	87
"	1:10	113
Sulfaguanidine	1:1	114
Succinyl sulfathiazole	1:1	113
Neoprontosil	1:1	137

Effect of Sulfanilamide and PABA in Different Concentrations. Inasmuch as most of the previous work on the mode of action of sulfonamides has been done with sulfanilamide, the effect of sulfanilamide and PABA separately and together in various concentrations was next studied more intensively.

Sixty experiments were performed with each

[8] Macht, D. I., and Livingston, M. B., *J. Gen. Physiol.*, 1922, **4**, 573.

[9] Macht, D. I., and Grumbein, M. L., *Am. J. Botany*, 1937, **24**, 457.

TABLE II.
Growth of *Lupinus albus* Seedlings

In aqueous solutions of sulfanilamide		In aqueous solutions of PABA	
Concentration × 10,000	Index of growth	Concentration × 10,000	Index of growth
1:1	68	1:0.1	19 (plasmolysis)
1:2.5	73	1:1	60
1:5	77	1:2	64
1:10	94	1:2.5	73
1:20	100	1:5	77
1:25	106	1:10	80
1:50	110	1:20	82
		1:25	84
		1:50	88
		1:100	107
		1:125	110

TABLE III.
Growth Indices for Combination Experiments with Lupinus Seedlings.

Experiment	PABA dilution	Sulfonamide dilution	PABA and sulfa (equal parts)
I	25,000 ×	250,000 ×	57%
G.I.	70%	94%	
II	500,000 ×	250,000 ×	74%
G.I.	86%	100%	
III.	500,000 ×	25,000 ×	51%
G.I.	82%	81%	
IV	250,000 ×	10,000 ×	50%
G.I.	84%	71%	
V	25,000 ×	25,000 ×	63%
G.I.	72%	78%	
VI	250,000 ×	250,000 ×	53%
G.I.	78%	100%	
VII	25,000 ×	250,000 ×	72%
G.I.	72%	105%	
VIII	1,000,000 ×	1,000,000 ×	97%
G.I.	105%	107%	

drug and a summary of the results is shown in Table II. It will be seen that sulfanilamide exerts an inhibitory effect on growth in concentrations below 1:100,000 but that in weaker solutions it is mildly stimulating to growth.

With para-aminobenzoic acid, a diphasic action may also be noted; in suitable concentrations it stimulated root growth.

When various concentrations of the 2 drugs were used together 1:1 a definite *synergism* or potentiation was noted in every instance, not only when the drugs were dissolved in Shive solution, but also when they were dissolved in *water* without admixture of plant-physiological electrolytic salts. These data are obviously quite unlike those reported by observers who used mixtures of the same drugs on bacteria. (Table III.)

Comment. The PABA theory for the mode of action of sulfanilamide as advanced by the original workers, Lockwood,[10] Woods,[11] Fildes,[12] Bliss and Long,[13] and others, is probably the most satisfactory explanation so far offered. It is based on the cardinal fact that para-aminobenzoic acid antagonizes the action of sulfanilamide on bacteria and other organisms. Thus, Maier and Riley[14] found it to inhibit the sulfanilamide effect on plasmodia, and similar observations were made

[10] Lockwood, J. S., *J. Immun.*, 1938, **35**, 155.

[11] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[12] Fildes, P., *Brit. J. Exp. Path.*, 1941, **22**, 293.

[13] Bliss, Eleanor A., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 14.

[14] Maier, J., and Riley, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 152.

by Marshall, Litchfield, and White,[15] and by Seeler, Graessale, and Dusenberg.[16] PABA counteracts the inhibiting effect of sulfanilamide on cell division of the flagellate *Polytomella caeca*.[17] Similar antagonism is exerted by para-aminobenzoic acid on fresh water diatoms;[18] on pigment formation of *Pseudomonas seruginosa*;[19] on the effect of sulfanilamide on experimental *Lymphogranuloma venereum* virus,[20] and on some yeasts.[21]

It is therefore surprising that the influence of sulfanilamide and PABA combinations on higher plants such as *Lupinus albus* is diametrically opposite and the converse of antagonism.

This is of interest from the standpoint of general biology. In the earlier days of bacteriology it was generally held that bacteria are microscopic plants. In fact, older writers often resorted to yeast cultures for testing the antiseptic properties of drugs. In the light of our present findings the behavior of bacteria under the combined action of sulfanilamide and PABA resembles more the effects produced by these drugs on plasmodia and flagellates which are commonly classed as animal organisms. The results yielded by a study of *Lupinus albus* emphasize the uncertain demarcation between the lower animal and plant organisms. The present findings illustrate well the dangers of hasty and sweeping generalizations in pharmacology. It is hazardous to apply deductions from data of one species of zoo- or phyto-pharmacological test-objects to another, without experimental tests in support of such conclusions.

Summary. Pharmacological studies on root-growth of *Lupinus albus* seedlings under standardized and rigidly controlled conditions reveal that: (1) Of the 3 amino-benzoic acids the *meta* isomer is the most toxic, and the *para* isomer is the least toxic. (2) Para-aminobenzoic acid exerts a diphasic effect on root-growth: Very weak dilutions are growth-promoting, while stronger concentrations are growth-inhibitory. (3) Sulfanilamide also exhibits a similar action, some concentrations inhibiting growth while other concentrations promote it. (4) Similar findings were made with 6 other sulfonamides which were examined. (5) Combinations of sulfanilamide and para-aminobenzoic acid in every case exerted not an antagonistic, but on the contrary, a synergistic or potentiated depression on growth of *Lupinus albus* roots.

15 Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J., *J. Pharm. and Exp. Ther.*, 1942, **75**, 89.

16 Seeler, A. O., Graessale, O., and Dusenberg, E. D., *J. Bact.*, 1943, **45**, 205.

17 Lwoff, A., Nitti, F., Trefowel, Mme. J., et Hamon, V., *Ann. Inst. Pasteur*, 1941, **67**, 9.

18 Wielding, S., *Science*, 1941, **94**, 389.

19 Ungar, J., *Nature*, 1943, **152**, 22.

20 Findlay, G. M., *Brit. J. Exp. Path.*, 1940, **21**, 356.

21 Landy, M., and Dicken, D. M., *Nature*, 1942, **149**, 244.

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Cause of an Outbreak of Encephalitis Established by Means of Complement-Fixation Tests.*

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On 16 July, 1945, members of the staff of Naval Medical Research Unit No. 2 arrived on Okinawa to study an outbreak of encephalitis which had appeared among natives of the island during the first week in July. The danger that the encephalitis might interfere with military and naval operations in the Ryukyus made it imperative that the etiologic agent be identified as soon as possible so that proper control measures could be instituted.

* The Bureau of Medicine and Surgery of the Navy does not necessarily endorse the views set forth in this paper.