EFFECT OF COCAINE ON THE GROWTH OF LUPINUS ALBUS. A CONTRIBUTION TO THE COMPARATIVE PHARMACOLOGY OF ANIMAL AND PLANT PROTOPLASM.

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INTRODUCTION.

The effects of chemicals on plants have in some respects been studied very thoroughly and in other respects almost not at all. Those drugs or chemicals which have been intensively investigated in this connection are the various salts or more accurately speaking ions which are necessary for the nutrition of various plants. The vast amount of work in plant physiology which has been done on this subject of plant nutrition and plant metabolism has been probably more thorough than the analogous observations on animals. At any rate the plant physiologist is able to produce a more perfect nutritive solution for the growth of plants than the animal physiologist can for the study of animal organs or tissues. While this phase of plant chemistry has received all the attention demanded by its importance, the influence of other chemicals or drugs and poisons on plant life has been barely touched upon. Yet the few contributions along these lines which have appeared, emphasize its importance. If we define pharmacology[1] as some authors do, as ". . . . the study of the changes induced in living organisms by the administration in a state of minute division of such substances as do not act merely as foods" then the field of what we might term *phyto-pharmacology* is virgin soil. The effects of drugs or poisons on plants have been very little studied. The drugs which have perhaps received slightly more attention in this

[1] Cushny, A. R., A textbook of pharmacology and therapeutics or the action of drugs in health and disease, Philadelphia and New York, 4th edition, 1906.

respect are metallic salts. The effects of these on sprouting and growth have been studied by some authors such as Brenchley (1), Bokorny (2), and a few others. The deleterious effects of some gases have also been touched upon by some authors, such as Crocker and Knight (3), Crocker, Knight, and Rose (4), and others, and the effects of ether on flowers have been investigated by other plant physiologists (5). The influence of that most important class of drugs or poisons known as the alkaloids on the growth of plants, has, however, with the possible exception of nicotine (6) practically been left untouched.

In connection with the comparative study of the effects of cocaine and its decomposition products on various animal organs and tissues carried on by one of the authors, it was thought of interest to inquire into the effects of the same compounds on living organisms belonging to the plant kingdom and an investigation on the subject was accordingly undertaken with the kind cooperation of Professor Livingston of the Laboratory of Plant Physiology of this University. The results obtained were so interesting in themselves, and even more so as compared with the data obtained from animal work, that they are deemed worthy of publication.

Methods.

In the present investigation the effect on the growth of cocaine and its decomposition products were studied, in terms of elongation, of the roots of the seedlings of *Lupinus albus*. This lupine can be very easily germinated and the single straight root of the seedling can be readily measured. The procedure employed in the present experiments was as follows. The dry seeds were soaked over night in tap water at ordinary temperature. On the following day the swollen seeds were planted with the hilum downward in moist, finely ground sphagnum moss. The planted seeds are placed in a thermostat and left at a constant temperature of 20°. On the third day after planting, the roots of the seedlings are of convenient length for measurement and are ready for study. After recording the exact length of a root it is placed in an upright test-tube of hard glass containing nutrient solution, the seed resting on the upper edge of the tube. The solution employed was the so called Shive solution

which contains calcium nitrate, magnesium sulfate, and monopotassium acid phosphate (7). Such a solution was prepared by mixing 10.4 cc. of 0.5 molar solution of calcium nitrate, 30 cc. of 0.5 molar solution of magnesium sulfate, and 36 cc. of 0.5 molar phosphate, with distilled water sufficient to make 1 liter. The normal growth of the lupine rootlets was studied by immersing the seedlings in a mixture of normal Shive solution with an equal part of distilled water. The effect of cocaine and other drugs was studied by dissolving chemicals in distilled water and mixing such drug solutions with equal parts of the normal Shive solution. After measuring accurately the length of each root and placing the seedlings in the control and drug solutions, the whole was again put in the incubator and left at a constant temperature of 20°C., and the effect of various chemicals on the growth of the roots was determined on the following day; that is, at the end of 24 hours. Ten seedlings were placed in the control solution, of one-half Shive solution and one-half water, and ten seedlings were employed for the study of each drug solution. In most of the experiments the volume content of the test-tubes used was about 10 cc. In the case of a few rare and valuable chemicals test-tubes of shorter length, holding about 5 cc., were occasionally employed.

The influence of the following substances, on the growth of lupine roots was investigated: cocaine hydrochloride, sodium benzoate, methyl alcohol, methyl benzoate, ecgonine hydrochloride, benzoyl ecgonine, and various mixtures of these drugs. It is well known that the cocaine molecule can be easily decomposed by hydrolysis, yielding *ecgonine*, *methyl alcohol*, and *benzoic acid*. The anesthetic properties of cocaine in animals are dependent on the chemical union of these three components. A simple mixture of the three will not give the same pharmacological effects as the chemical combination in the form of the cocaine molecule. This is true not only in regard to the anesthetic properties of cocaine but, as previously shown by one of the authors with various collaborators, also holds good in regard to the action of cocaine on the central nervous system (8), on skeletal muscle (9), and on smooth muscle. It was for this reason that it was deemed desirable to inquire into the effect on the lupine root, not only of cocaine itself but also of its various decomposition products.

All of the above substances were studied in various concentrations, the object being to ascertain, in the first place, whether they were toxic to the roots at all and if so to determine their limits of toxicity; that is, the minimal concentrations of the drug which produce a retardation in growth or other toxic effects.

EXPERIMENTAL.

Effect of Cocaine.—Cocaine hydrochloride was employed in the experiments, the chemically pure salt was dissolved in distilled water,

TABLE I.

Relation between Concentration of Cocaine Hydrochloride and Growth of Lupine Roots.

Concentration of cocaine hydrochloride.		Growth increment, for 24 hrs., expressed as percentage of corresponding increment in control test.
mol per liter	*per cent*	
0.2, 0.1, 0.08, 0.06	6.8, 3.4, 2.7, 2.0	No growth.
0.05	1.7	64
0.01	0.34	89
0.005	0.17	100
0.001	0.03	100
0.0005	0.17	100
0.0001	0.003	119, 93
0.00005	0.0017	80, 95
0.00001	0.0003	82, 121
0.000005	0.00017	91, 138
0.0000005	0.000017	90, 116
0.0000001	0.000003	100

and various concentrations of the solution were mixed with equal parts of normal Shive solution, the plants being immersed in the mixture. The results of the experiments are shown in Table I. In the first column are indicated the concentrations of the cocaine hydrochloride expressed as molar, and in the second column are given the equivalents of these concentrations in terms of per cent by weight. In the third column the mean growth increment of the seedlings in the cocaine solutions is indicated as compared with the corresponding normal or control increment, for 24 hours. It will be seen that growth of these roots is affected only by rather strong solutions of cocaine.

The minimal concentration required to inhibit growth completely was 0.06 molar, or about 2 per cent. Solutions slightly more dilute (*e.g.* 0.05 molar, or 1 per cent, allowed a growth increment of 64 per cent), and still weaker solutions did not impair the growth at all. In some of the experiments, indeed, as for instance in concentrations of 0.0000005 molar and 0.0001 molar, there was even a suggestion of a stimulation in growth. This was not, however, definitely established, because of lack of cocaine. The significance of the toxic dose of cocaine for the lupine as compared with that of the same drug for animal tissues will be discussed below.

Toxicity of Ecgonine and Benzoyl Ecgonine.—Cocaine on being hydrolyzed readily yields the base ecgonine. Neither ecgonine nor

TABLE II.

Relation between Concentration of Ecgonine Hydrochloride and Growth of Lupine Roots.

Concentration of ecgonine hydrochloride.		Growth increment, for 24 hrs., expressed as percentage of corresponding increment in control test.
mol per liter	*per cent*	
0.005, 0.0025	0.11, 0.055	No growth.
0.001	0.022	78
0.0001	0.0022	83
0.00005	0.0011	85
0.00001	0.00022	100
0.000005	0.00011	100

benzoyl ecgonine exhibit the characteristic pharmacological properties of cocaine in animals. The effects of these compounds on the growth of lupine are shown in Tables II and III. Table II gives the effects of various concentrations of ecgonine hydrochloride, while Table III exhibits the results of experiments with benzoyl ecgonine. It will be noted that the minimal concentration of ecgonine hydrochloride which produced complete inhibition of growth was 0.0025 molar, or 0.055 per cent. Weaker dilutions ranging from 0.001 to 0.00005 molar, inclusive, permitted growth, though considerably retarding it. In concentrations of 0.00001 molar no deleterious effect on the growth of the roots was evident. It is thus seen that ecgonine is more toxic to lupine roots than is cocaine itself.

An examination of Table III reveals the fact that the toxic dose of benzoyl ecgonine is larger than that of ecgonine itself; in other words the benzoyl ecgonine is less toxic than ecgonine. In order to inhibit growth completely, concentrations of 0.1 molar, or 3.29 per cent, were required. A concentration of 0.1 molar, and lower ones, did not retard

TABLE III.

Relation between Concentration of Benzoyl Ecgonine and Growth of Lupine Roots.

Concentration of benzoyl ecgonine.		Growth increment, for 24 hrs. expressed as percentage of corresponding increment in control test.
mol per liter	*per cent*	
0.1	3.29	No growth.
0.08	2.632	31
0.04	1.316	77
0.01	0.329	100

TABLE IV.

Relation between Concentration of Methyl Alcohol and Growth of Lupine Roots.

Concentration of methyl alcohol.		Growth increment, for 24 hrs., expressed as percentage of corresponding increment in control test.
mol per liter	*per cent*	
1.5	4.8	No growth.
1.0	3.2	27
0.5	1.6	88,73
0.3	0.96	1.05
0.1	0.32	1.19, 1.16, 1.16
0.05	0.16	1.28
0.01	0.03	76
0.005	0.02	75
0.001	0.003	97

growth at all. It is evident that benzoyl ecgonine, unlike ecgonine itself, is somewhat less toxic than cocaine.

Effect of Methyl Alcohol.—The results obtained with methyl alcohol are shown in Table IV. This drug was found to be but little toxic for lupine roots. It required a concentration of 1.5 molar, or 4.8

per cent, to kill the plant, while 1 molar, or 3.2 per cent, gave a growth increment of 27 per cent, or as large as occurred in the controls. Concentrations of 0.1 molar, or 0.32 per cent, gave results suggestive of a stimulation of root elongation.

Effect of Sodium Benzoate and Methyl Benzoate.—The results obtained with these compounds were most interesting, as shown in Tables V and VI. It was found that these esters were both very toxic for lupine roots. Solutions of sodium benzoate as dilute as 0.305 molar, or 0.007 per cent, completely inhibit growth, while methyl benzoate produced complete inhibition in concentrations of 0.0001 molar, or 0.014 per cent.

Effect of Some Mixtures.—Inasmuch as combinations of drugs in animal experiments often give synergistic or antagonistic results, experiments were made with various mixtures of some of the compounds above considered. Four sets of mixtures were prepared and labeled respectively A, B, C, and D. Mixture A consisted of sodium benzoate, methyl alcohol, and ecgonine hydrochloride, the quantity of each used being one-third of the individual lethal dose. Mixture B consisted of sodium benzoate, methyl alcohol, and ecgonine hydrochloride, the quantity of each used being one-third of the individual lethal dose. Mixture C contained one-half of the lethal doses of ecgonine hydrochloride and methyl benzoate, respectively. Mixture D contained one-half of the lethal doses of benzoyl ecgonine and methyl alcohol, respectively. These mixtures were employed in some experiments in their original concentrations, and in other experiments dilutions of these mixtures were tried. It was found, as might have been expected, that Mixture A produced death of the plants. It was furthermore found that, when Mixture A was diluted to one-half the original concentration, it still produced death; in other words, it gave a synergistic effect. Mixture B was also found to be more toxic than was to be expected from a simple summation of its individual components. On the other hand, Mixtures C and D were found to be slightly less toxic than the arithmetical sum of their component effects; in other words, they suggest a slightly antagonistic action of the components against each other.

TABLE V.

Relation between Concentration of Sodium Benzoate and Growth of Lupine Roots.

Concentration of sodium benzoate.		Growth increment, for 24 hrs., expressed as percentage of corresponding increment in control test
mol per liter	*per cent*	
0.174	2.5	No growth.
0.087	1.25	" "
0.07	1.00	" "
0.04	0.625	" "
0.017	0.25	" "
0.0087	0.125	" "
0.003	0.05	" "
0.0028	0.04	" "
0.002	0.03	" "
0.0015	0.022	" "
0.0014	0.02	" "
0.001	0.0144	" "
0.0005	0.007	" "
0.0003	0.004	11
0.0001	0.001	56.2, 62
0.00005	0.0007	67, 66
0.00001	0.0001	11.1
0.000005	0.00007	0.83
0.000001	0.00001	0.97

TABLE VI.

Relation between Concentration of Methyl Benzoate and Growth of Lupine Roots.

Concentration of methyl benzoate.		Growth increment, for 24 hrs., expressed as percentage of corresponding increment in control test.
mol per liter	*per cent*	
0.001, 0.0001	0.136, 0.014	No growth.
0.00005	0.007	13
0.00001	0.0014	30
0.000005	0.0007	74
0.000001	0.00014	79
0.0000005	0.00007	90,90
0.0000001	0.000014	75,93
0.00000005	0.000007	94

DISCUSSION.

The results obtained with cocaine and its various other compounds on the growth of lupine roots are interesting in themselves, but they become much more so when compared with the effects of the same substances on animal organs and tissues. The results of such a comparison are shown in Table VII. In this table the first column indicates the drugs used, the second column expresses the minimal lethal doses of the same for *Lupinus albus*, while the other columns indicate the corresponding effects on animal tissue. In Column 3 are shown the influence of cocaine and its decomposition products, as well as various other mixtures, on the central nervous system. These results are taken from a study by Macht and Bloom (8) concerning the effects of cocaine on the behavior of white rats in the circular maze. In the fourth column the local anesthetic effects of the various drugs are shown. In Column 5 the effects of cocaine and the other drugs on skeletal muscle are described, the data being taken from Kubota and Macht (9). In Column 6 the toxicity of the various compounds for smooth muscle is indicated. These figures are taken from the as yet unpublished studies of the effects of cocaine on the smooth muscle of the bladder and ureters carried on by Macht and Satani. Finally, the lethal doses of cocaine for cats are given in the last column, the figures being taken from Heffter.[2]

It will be noted that, whereas cocaine is by far the most toxic of the substances studied for animals, it is not nearly so toxic for plants. The lethal dose of cocaine for cats is given as 0.02 to 0.04 gm. per kilo, whereas to produce complete inhibition in the growth of the plant a 2.04 per cent solution is required. Ecgonine hydrochloride is quite toxic for animal tissues and, indeed, in the case of skeletal muscle it paralyzed the contractions of the same in 45 minutes, an effect slightly more toxic than that of cocaine (55 minutes). The lethal dose of ecgonine hydrochloride for lupine roots was comparatively very much smaller than that of cocaine, a concentration of 0.55 per cent being sufficient to kill. Benzoyl ecgonine was much less toxic for lupine roots than ecgonine itself, a result which ran parallel to the compara-

[2] Poulssen, E., in Heffter, A., Handbuch der experimentellen Pharmakologie, Berlin, 1920, ii, pt. 1, 145 ff.

TABLE VII.

Comparison between Effects of Cocaine and Related Substances on Lupine Roots, Animal Tissues, etc.

Substance. (1)	Minimal lethal dose for lupine roots. (2)		Effect on central nervous system. (3)	Local anesthetic effect. (4)	Time for paralysis of skeletal muscle. (5)	Effect on smooth muscle. (6)	Lethal dose for cat. (7)
	M	*per cent*			*min.*		*gm. per kilo*
Cocaine hydrochloride ..	0.06	2.04	Depression.	Effect was obtained	55	1:20,000 1:5,000	0.02–0.04
Ecgonine hydrochloride .	0.0025	0.055	None.	None.	45	1:20,000	Over 2.0.
Benzoyl ecgonine.....	0.1	2.5	"	"	Very slight toxicity.	1:750	1.7
Sodium benzoate	0.0005	0.007	"	"	No toxicity.	No effect observed.	0
Methyl alcohol.....	1.5	4.8	"	"	" "	1:50	4.7
Methyl benzoate.....	0.0001	0.014		"			
Mixture A.....	(Synergism.)		None.		Toxic.		
Mixture B.....	(Potentiate.)		"				
Mixture C.....	(Antagonism.)		"				
Mixture D.....	(")				Very slight toxicity.		

tive toxicity of the two for animals. Methyl alcohol was found to be not very toxic for either animal tissues or lupine roots. The most remarkable results obtained, however, were those with sodium benzoate. This salt is very slightly toxic indeed for animal tissues, so that it is employed as a food preservative, yet it was found to be the most toxic of all the compounds studied for lupine roots. It required a concentration of only 0.007 per cent to kill the plants. Methyl benzoate was also very toxic, being second only to sodium benzoate in that respect.

The reason for the remarkable difference just pointed out, in the toxicity of cocaine for animal and for plant tissues, is as yet unknown. The effects of the drugs studied are certainly not attributable to a simple change in the hydrogen ion concentration of the culture media, for experiments made by the authors showed that the Shive solution may be made more or less acid or alkaline without appreciably affecting the growth of the lupine roots. The value of the pH of the normal Shive solution here used was 6.4. More acid and less acid solutions were prepared by varying the quantity of potassium phosphate used, and it was found that the hydrogen ion concentrations varied from 4.4 to 7.2. Within these limits the growth of lupine roots was very little affected.

SUMMARY.

1. The effects of cocaine and its decomposition products were studied on the growth of the young roots of *Lupinus albus*.

2. The results obtained were compared with similar experiments on animal tissues.

3. It was found that, while cocaine is the most toxic of these compounds studied for animal tissues, it was of comparatively low toxicity in respect to its effect on the growth of roots. On the other hand, sodium benzoate, being practically non-toxic for animals, was the most toxic of the compounds studied for the plant roots.

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