THE EFFECT OF ALKALOIDS ON REGENERATION IN THE SCARLET RUNNER BEAN.

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The difference in the rates of growth is undoubtedly one of the most important problems in the study of regeneration at the present time. The problem is now being attacked by a number of zoologists, who study this question from the point of view of the relation of the rate of regeneration to either the degree of injury, or the frequency of injury, or the levels at which the organism is injured.

Although zoologists agree as to what phenomena are to be described under the head of "regeneration," there is still no concensus of opinion with regard to plants; and while Pfeffer (6), for instance, would limit the term regeneration to those cases only "in which an organ replaces a portion of itself which has been removed" (p. 167), Goebel (2) and others contend that phenomena of regeneration imply also a development of dormant or latent buds present before injury.

McCallum (4) finds that of all the plants under his observation there were scarcely any "in which these primordia developed without the removal of the shoot, and in every case in which the stem was cut off they developed."

In an investigation, (now in progress), upon the regeneration of animals, the attempt was made by the writer to study the problem from a new standpoint, that of the modifiability of the rate of regeneration under changed external conditions, with the hope of throwing some additional light upon this somewhat perplexing problem. It seemed desirable, in connection with these experiments upon animals, to test the method also on regenerating plants, especially since the subject of the rate of regeneration, so far as the writer's knowledge goes, does not seem to have been touched upon by botanists. It was important for the purpose in hand to obtain a plant in which the regenerative processes had already been investigated and which at the same time would be available for further experimentation. Such an object was found in the scarlet runner bean, a variety of Phaseolus multiflorus, on which McCallum (4) has done much valuable research, and ascertained many points of importance.

Omitting details, the method followed was briefly this: Seeds of the scarlet runner bean were germinated in sawdust until they had reached a height of 6 cm. The seedlings were then transferred to pint mason jars. Each jar was completely covered with black paper in order to protect the growing roots

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from the action of sunlight. There were two plants in each jar, held in place by means of perforated corks which had been previously paraffined. It may also be mentioned that the corks were subsequently paraffined to the jars so as to prevent any evaporation of water except that through the leaves.

The plants were subjected to the influence of a few alkaloids in solutions of various degrees of concentration in order to determine the effect which these substances would produce upon the rate of regeneration of new shoots. The following alkaloids were used: Sulphates of atropine and strychnine, pilocarpine hydrochloride and digitalin, each in a (a) 0.01%, (b) 0.001%, and (c) 0.0001% aqueous solution. Each jar contained 400 cc. of the solution; distilled water was used for the controls. The water in all jars was changed at intervals, and carefully weighed. The full series of plants was kept always in the same place in the university greenhouse so as to insure equal conditions of light, temperature and air currents.

The stems were cut off with sharp scissors very near the base the second day after the plants had been transferred to the mason jars. The rudiments of new stems appeared shortly afterwards in the axils of the cotyledons.

It is known that a close relationship exists between the quantity of transpiration and the amount of growth for a given length of time (3), and that the index of transpiration is usually relied upon in comparing the rate of growth under varied conditions. Since transpiration is a continuous physiological process in living plants it was hoped that such data might aid in obtaining an insight into the physiological condition of plants regenerating under the influence of the various alkaloids. Unfortunately these data do not always prove to be quite a reliable basis for comparison of the actual amounts of regeneration for definite periods; they were therefore checked up by other data; as for instance, the weight of plants. The failure of the indices of transpiration to offer a solid ground for the comparative study of the rates of regeneration in this particular case of the scarlet runner bean is probably due in a large measure to the circumstance that the total surface area of the leaves, the number and form of which differed during regeneration almost with each plant, is not sufficiently uniform; and consequently the amount of water lost through transpiration does not always correspond to the real rate of regeneration. The following table contains data on transpiration:

TABLE I.

Date—February 27, to March 15, 1908.

Solution	Transpiration	
	Total	Daily Average
Control I	69.95 grms.	4.12 grms.
Control II	58.65 "	3.45 "
Strychnine 0.01%	43.95 grms.	2.59 grms.
" 0.001%	93.10 "	5.48 "
" 0.0001%	91.20 "	5.37 "
Digitalin 0.01%	64.60 grms.	3.80 grms.
" 0.001%	90.65 "	5.33 "
" 0.0001%	123.30 "	7.25 "
Pilocarpine 0.01%	108.90 grms.	6.46 grms.
" 0.001%	61.05 "	3.59 "
" 0.0001%	108.55 "	6.39 "
Atropine 0.01%	65.25 grms.	3.84 grms.
" 0.001%	61.90 "	3.64 "
" 0.0001%	80.35 "	4.73 "

The illustrations (Figs. 1–4) which are reproduced from photographs of plants that had been regenerating about 8 days after the epicotyls were cut off, will serve for comparing the actual sizes attained by the different plants. Reading from left to right the arrangement of the cultures in the photographs is in all cases as follows: control, solutions *a, b, c*. From these figures it will be seen that the rate of regeneration varies both with the nature of the medium and also with the strength of the solution in which the plants regenerated. In pilocarpine (Fig. 1) the plants developed more luxuriantly than the controls and the greatest acceleration is shown in both the stronger and the weaker solution (0.01% and 0.001%). In solutions of digitalin (Fig. 2) the greatest acceleration took place in the weaker solution, while the regeneration was slower in the strong solution (0.01%). Atropine (Fig. 3) and strychnine act as stimuli only in very weak solutions (0.0001%). In extremely attenuated solutions of atropine and strychnine, such as were obtained by means of filtrating these solutions through lamp black or through calcium carbonate (1), the process of regeneration was augmented to a remarkable degree. Solutions of strychnine (Fig. 4) act differently upon different plants, but the greatest stimulation is caused invariably by the weaker solutions. Stronger solutions, though they may stimulate growth at first, soon become injurious to the plant.

At the end of twelve days after the operation the differences in size of the regenerating plants became much more pronounced than those seen in the figures shown here.

In the following table are given the data concerning the green and dry weight of the plants.

TABLE II.

Solution	0.01%		0.001%		0.0001%	
	Weight of plts. in grs.		Weight of plts. in grs.		Weight of plts. in grs.	
	Green	Dry	Green	Dry	Green	Dry
Strychnine	2.25	0.56	3.50	0.88	5.00	1.25
Digitalin	3.75	0.94	4.80	1.20	7.70	1.93
Pilocarpine	6.65	1.64	5.05	1.26	5.95	1.49
Atropine	2.95	0.74	2.25	0.56	3.25	0.81
Control I	2.40	0.60				
Control II	1.85	0.46				

It may be seen from this table that the inference which should be drawn from records of the weight of the regenerated stems practically coincides with data obtained from the study of their transpiration and actual size.

On the 15th of March, i. e., seventeen days after the operation the regenerated stems were cut off once more with sharp scissors near their proximal ends. The object in performing this experiment was to find out how the plants would behave in regeneration after a second operation. In a previous work on regeneration in the fresh water oligochaete, Lumbriculus, (5a) it was pointed out that the rate of regeneration in that animal decreases after successive operations, so that if we designate the rate of regeneration after the first operation by a unit, the rate of regeneration after a second operation would be only one-half of a unit, and one-fourth of a unit after the third operation, the length of time during which the animals are allowed to regenerate being, of course, the same in all three cases. Zeleny (8), on the other hand, finds that

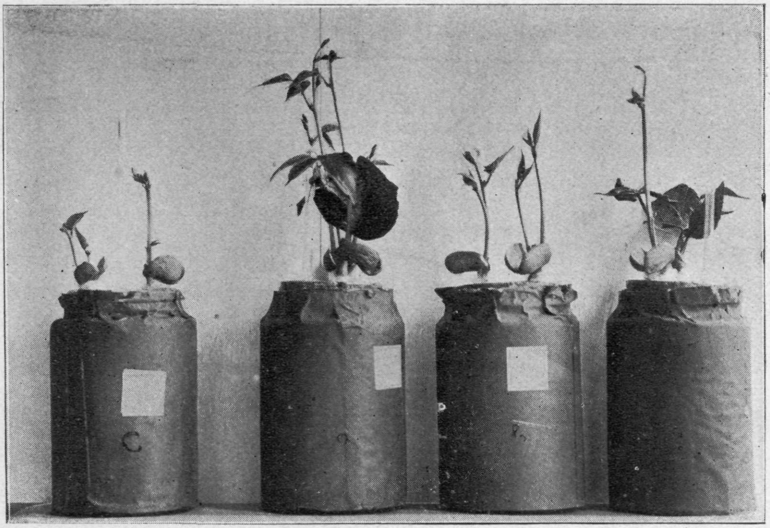

Fig. 1. Phaseolus in pilocorpine solution.

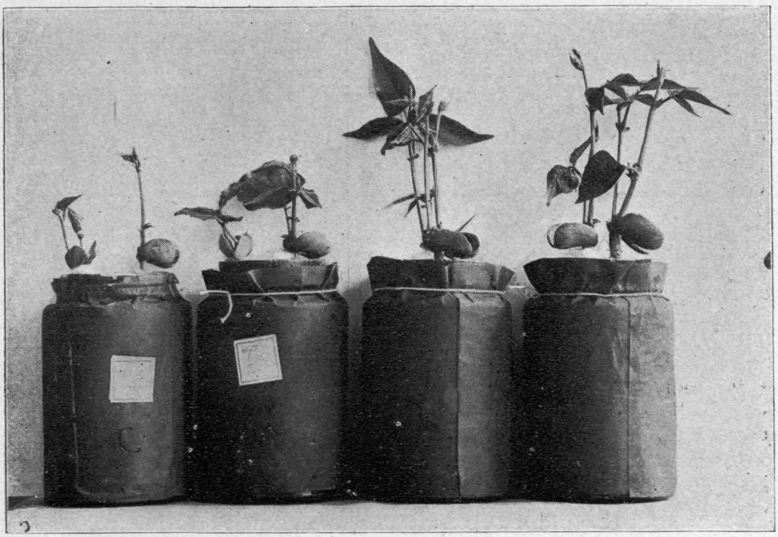

Fig. 2. Phaseolus in digitalin solution.

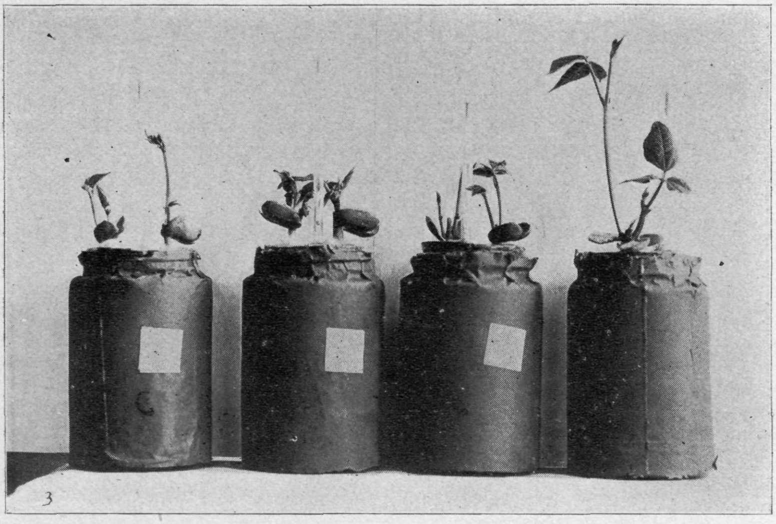

Fig. 3. Phaseolus in atropine solution.

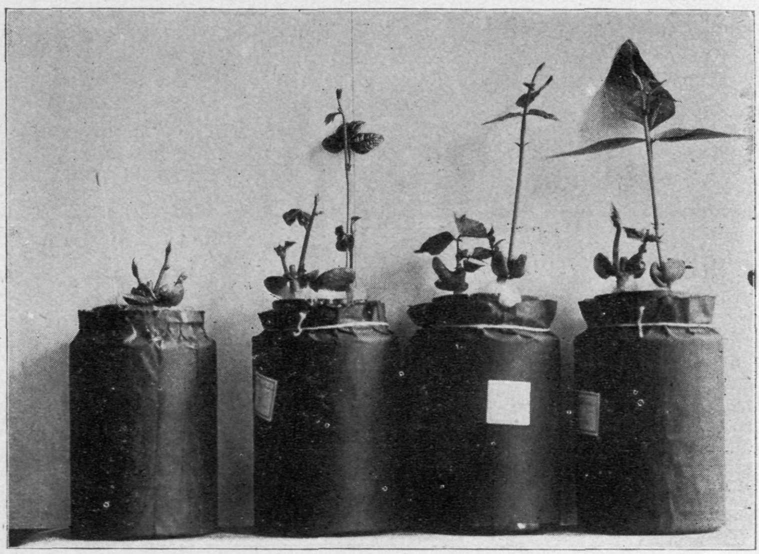

Fig. 4. Phaseolus in strychnine solution.

in the Scyphomedusan, Cassiopea xamachana, "there is a well marked difference between the second and the first regeneration in favor of the former."

A rather long interval followed upon the second operation, before the plants commenced to regenerate again. The new stems were now growing from the axils between the stump of the old epicotyl and the stumps of the regenerated stems that were cut off March 15; and also from the axils between the latter ones and the cotyledons. In other words, in place of the two stems which had regenerated after the first operation there were regenerating now four stems, as may be seen from the accompanying diagram. It should be mentioned, however, in this connection, that the four stems have not always regenerated equally well from each plant.

The following Table III contains data concerning transpiration, weight of all the regenerated material and also extracts from protocols, relating to the condition of the plants:

TABLE III.

March 15 to April 6, 1908.

Nature of Solution	Transpiration		Weight of regener. tissue	Remarks March 28
	Total	Daily average		
	Grms.	Grms.	Grms.	
Control	48.70	2.21	0.75	
Strychnine 0.01%	14.50*	1.11	.....	Dead
" 0.001%	31.15	1.42	0.30	Regen. slightly
" 0.0001%	48.80	2.22	1.05	Regen. well
Digitalin 0.01%	42.50	1.48	0.75	Reg. stems small
" 0.001%	74.25	3.38	1.80	Reg. well
" 0.0001%	108.65	4.94	2.10	Reg. stems large
Pilocarpine 0.01%	62.40	2.84	1.75	Stems reg. well
" 0.001%	22.50	1.02	0.20	Stems small
" 0.0001%	96.75	4.40	1.90	Stems quite large
Atropine 0.01%	39.15	1.78	0.60	
" 0.001%	42.45	1.93	0.85	Stems regen. at fairly good rate
" 0.0001%	53.55	2.44	0.95	

*Total transpiration for 13 days only; the plants died soon afterwards.

From this table it will be observed that strychnine, especially in the stronger concentration acted poisonously upon the plants, checking rather than stimulating their regenerative power. Even a superficial examination of the Tables I and III will leave no room for doubt as to the very large difference between the first and the second regeneration in favor of the former. And in spite of the fact that after the second operation the plants had been regenerating for a longer period of time, the regenerated

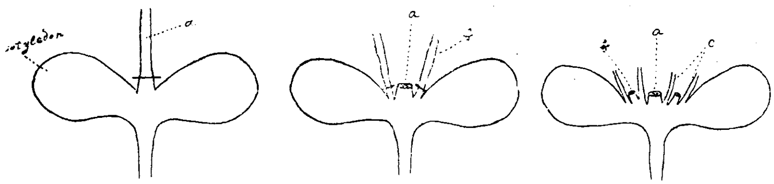

Fig. 5. Diagram of decapitated plants; *a*—old epicotyl, *b* and *c*—regenerating stems.

stems were by far smaller than those regenerated after the first operation. That the difference is not to be attributed to an exhaustion of food materials was evident because the cotyledons were still of a large size. Furthermore, McCallum (4) demonstrated in a series of ingenius experiments that food is not a necessary factor in regeneration of Phaseolus, and that the plants regenerate even with the cotyledons removed.

CONCLUSION.

In summing up the facts presented in this note it may not be amiss, perhaps, to discuss briefly their relation to facts obtained in other similar studies. It was shown in the foregoing that alkaloids, such as pilocarpine, atropine, strychnine and digitalin exert a stimulating influence upon regenerating plants, increasing the rate of regeneration. Yasuda (7) found from his study of the effect of alkaloids upon moulds that "the moulds generally grow better in the solutions which contain alkaloids than in the normal control-solution." (p. 82.) He also found that strychnine produced no poisonous action on the moulds until the limit of saturation was reached (about 2.5%). Plants behave somewhat differently in this respect from animals. In a recent work on the effect of alkaloids upon the early development of eggs of the sea-urchin, Toxopnenstes variegatus, conducted at the Bermuda Biological Station (5c), atropine, strychnine and digitalin were found to inhibit the developmental process, the last two substances being so much toxic that normal development was possible only in very dilute solutions. Neither did pilocarpine stimulate or accelerate to any marked degree the development of the eggs of this sea-urchin, although the literature contains a record to the contrary with reference to the eggs of Asterias Forbesii. With the exception of the stronger solution of strychnine, Phaseolus was able to live in concentrations which would prove fatal to animals.

The effect of the action of these alkaloids upon plants varies both with the nature and with the strength of the solution, but on the whole a general rise and intensification of the vital processes is seen as, for instance, in the augmentation of the function of transpiration, and in the higher rate of regeneration as compared with plants not subjected to the influence of stimulating agents. It seems therefore, legitimate to assume that there exists an intimate relation between the rate of regeneration and the physiological condition of the regenerating organism.

Concerning the rate of regeneration after consecutive operation it is obvious, from the facts cited above, that after the second operation there is a considerable decrease of the power of regeneration and, consequently, a slowing down of the process, as well as a considerable lengthening of the period which intervenes between the operation and the first appearance of regenerated tissue.

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It gives me pleasure to express here my gratitude to Dr. A. Dachnowski for much friendly assistance in this work.

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