ONCOLITHIC ACTIONS OF SOME POLYPLOIDOGENIC AGENTS (ACENAPHTENE, PODOPHYLLIN AND VITAMIN K) ON „PHYTOCARCINOMATA".

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With 2 Tables in the text and 7 Figures on Plates III—IV.

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INTRODUCTION.

The curative effects of the polyploidizing agent, colchicine, on experimental "phytocarcinomata", induced by *Bacterium tumefaciens*, were the subject of previous communications (Havas, 1937, 1937/a, 1939, 1940). This oncolithic effect of the alkaloid was confirmed later by several investigators (Brown 1939, Solacolu and Constantinesco 1939, Dermen and Brown 1940, 1940a, see also Levine 1945). The curative effects reported by these authors, by means of the local application of the drug on the tumours, were far superior to those obtained by myself through a general treatment of the plants. They amounted, in fact, practically, to a complete sterilization of the pathologic structures.

Encouraged, no doubt, by such results, Brown (1942) has attempted tumour reduction by means of several other chemicals known to elicit chromosome doubling. The most important of these was *acenaphtene,* the polyploidogenic effect of which was discovered by Kostoff (1938). Brown applied the chemical, mixed with lanolin or dissolved in dioxan, topically, painting with it the surface of the tumours. She found that while „3% acenaphtene in dioxan did not kill marigold tumours, young or old, it killed Paris daisy tumours. Any inhibition of growth of marigold tumours by the action of acenaphtene dissolved in dioxan was usually overcome...".

Previous experiments of the author (HAVAS, 1946, HAVAS and FELFÖLDY 1947) having demonstrated the surprisingly powerful and protracted influence of the vapours of acenaphtene, in oligodynamic concentrations, on actively growing tissues and organs, the object of the experiments to be outlined was to ascertain to what extent the sublimated particles of the drug would act in a preventive treatment, using at the same time a technique differing entirely from that of BROWN, on other meristematic structures, such as tumours.

Other experiments of a rather preliminary nature, owing to the novelty of the substances tried, were undertaken to test the eventual oncolithic actions of the newly discovered polyploidizing agents: podophyllin and vitamin K (2-methyl-1, 4 naphtaquinone).

Podophyllin is the resin of the fern *Podophyllum,* several varieties of which are known. It is claimed that among its ingredients podophyllotoxin (C_{22} H_{22} O_8) is the active principle from the pharmaceutical and therapeutic point of view (CZAPEK 1925, ISSEKUTZ 1941). Whether this is also the constituent which elicits the karyokinetic effects, remains to be elucidated.

KING and SULLIVAN (1947) have described the similarities of the cytological effects of the drug to those elicited by colchicine, when topically applied to *condyloma acuminatum.* SULLIVAN and WECHSLER (1947) have, moreover, pointed out the analogies of the action of podophyllin and colchicine on the meristematic tissues and cells of the root tips of *Allium cepa.* CORNMAN obtained, on the other hand, blocked mitoses in mouse sarcoma, and other colchicine effects, including polyploidy (private communication).

As to vitamin K, the polyploidizing and other colchicine-mimetic effects of this chemical, including tumour induction in plants, were shown by NYBOM and KNUTSON (1947) and by HAVAS (1947). This last has also observed (l. c.) the stimulation of the oncogenic action of colchicine on wheat seedlings when vitamin K was added to the alkaloid. In view of the double action of colchicine, oncogenic and oncolithic, (similar to that of many carcinogenic agents), the hypothesis of analogous action of vitamin K did not seem too far-fetched, and deserved a trial.

MATERIALS AND METHODS.

1. Acenaphtene. 104 potted tomato plants *(Lycopersicum esculentum* L. var. *„Üstökös")* were used in this experiment in greenhouse conditions. Of these plants 52 of approximately equal height (av. 11 cms.) and development were cut back to approximately 8.5 cms. and their leaves cut off. The reasons for this decapitation and mutilation were partly the observation that plants thus treated respond more readily to acenaphtene (HAVAS and FELFÖLDY 1947), and partly the necessities of the experimental technique to be used. The other implications of this treatment will be discussed in the last chapter. Acenaphtene was administered to 26 of these plants by the following method: 0.05 mg acenaphtene were dissolved in 0.5 ml ether in test tubes of ca 30 ml capacity. The tubes were then shaken until the ether evaporated, depositing the crystals of the drug on the walls of the tubes. These were then slipped on the stumps of the decapitated plants and the crystals were left to act on them by sublimation for 32 hours. Only a fraction of the drug had evaporated during this time, as shown by the quantity of crystals still coating the walls of the test tubes when these were taken off. The stumps of the 26 decapitated control plants were enclosed in similar glass tubes previously rinsed with ether and left on the plants, as above, for 32 hours.

16 plants of each of the decapitated groups, acenaphtene treated and untreated, were then inoculated with the tomato strain of *Bacterium tumefaciens* of the Pasteur Institute of Paris by means of 5 equi-distant pricks along the stem of each plant. The total number of inoculations was, in consequence, 160. The uppermost inoculation was made in the wound callus in process of formation after the decapitation. The remaining 10—10 decapitated plants, both acenaphtene-treated and untreated, were left as controls for discerning the various physiological syndroms of the inoculations, as compared with the effects of acenaphtene administered alone. Further controls were: 16 inoculated but undecapitated plants and 36 plants which were neither inoculated nor decapitated. 10 more uninoculated and undecapitated plants were treated with acenaphtene but as, on account of their bulk, bell-jars instead of test tubes had to be used for their treatment, no direct comparisons can be made with them.

2. Podophyllin. 15 castor oil plants *(Ricinus communis* L., var. *inermis* MAUTHNER) were inoculated with the *Chrysanthemum* strain of the Pasteur Institute of Paris, by means of 7 equi-distant pricks

(105 in all) along the stem of each plant. When the tumours (81 in all) had developed sufficiently to present an assortment of widely differing sizes (see foll. chapter) the plants were divided into two lots, each bearing approximately the same number of tumours, of approximately the same size. The tumours of one of these groups were painted twice with a mixture of podophyllin (CLAYTON and EDWARDS) in the proportion 1:1000, and a third time (this having run out), with podophyllin of Hungarian origin, in the proportion of 1:500. The control tumours were treated topically with lanolin alone. 10 more plants of the same variety, and similarly inoculated, were left entirely untreated. 5 *Ricinus sanguineus* plants, with a total of 17 tumours, were inoculated and treated with podophyllin, as above.

The effects of podophyllin were also tried on the tumours of tomato plants (var. *„Üstökös")* inoculated with the above tomato strain of *B. tumefaciens,* as above. The 38 tumours thus induced, distributed on 10 plants, were painted with podophyllin, like the castor oil plant tumours, and 42 tumours, distributed on 10 other plants, were treated with lanolin alone, to serve as controls.

3. Vitamin K. The effects of the vitamin were investigated on the tumours of *Pelargonium zonale* as induced by the above tomato strain of the pathogenic organism. The chemical was mixed with lanolin in the proportion of 1:1000 and applied directly on the tumours, as above. Three treatments were given. The control tumours were painted with lanolin alone. 44 plants were used in all. 25 tumours on 10 of these were treated with the vitamin and 20 tumours distributed on 10 other plants with lanolin alone. 22 plants inoculated as mentioned were left without any treatment, to serve as supplementary controls.

OBSERVATIONS.[1]

1. Acenaphtene. The first signs of tumour induction were noticed 13 days after the inoculations, and these only on the control plants, 24 days after the inoculations the number of tumours of the decapitated control plants was 65, while the decapitated acenaphtene plants produced only 7. The undecapitated and untreated controls showed 76 tumours.

[1] This account being mainly concerned with the effects on tumour growth of the agents tried, the details and statistical analysis of their growth effects and of their hormonal and polyploidizing influence will be published separately.

It was noticed at the same time that both the decapitated and undecapitated acenaphtene plants had suffered seriously from the emanations, as could be judged by comparing their rate of growth with that of both the inoculated and uninoculated decapitated controls. On the other hand, it could be ascertained that the mere fact of tumour growth did not cause an inhibition of straight growth, for the growth rate of the decapitated and inoculated, or decapitated and uninoculated acenaphtene plants was very nearly the same, and so was the growth rate of the inoculated and uninoculated controls which were not subjected to the vapours of acenaphtene. The decapitated acenaphtene plants, however, recovered and caught up quickly enough with the decapitated untreated controls, and even with the controls which were not cut back. Table I. shows the total height and weight of the treated plants and controls calculated per 16 plants in each group. It also shows that a resumption of tumour growth coincided with the resumption of the growth of the decapitated acenaphtene plants.

As regards the respective roles of decapitation, acenaphtene and bacterial infection in the induction of *polyploidy*, as judged by the size of the stomata of the leaves and other well known criteria, (such as their darker colour, increased thickness, and rough surface), Table I. furnishes the essential information. We shall discuss these important observations in the concluding chapter.

Both fasciated shoots and adventive roots (Figs. 1 and 2) were frequent in the acenaphtene-treated decapitated and inoculated plants. A phenomenon, of which we know no other example, was also observed on one of these plants, i. e., a shoot arising from a tumour, bearing three flowers, one of which developed into a miniature fruit (Fig. 3). While fasciations have been noticed before as a result of acenaphtene treatment (Havas and Felföldy l. c.), as well as stimulation of root initiation in wheat seedlings (Havas, unpublished), no causal connection can be suggested between the "adventive fruit" and the acenaphtene treatment.

At the end of the experiment, i. e. when about 20 % of the tumours showed signs of necrosis, they were cut off and weighed. Table I. shows the striking reduction in the weight of tumours of the the acenaphtene plants as compared with that of the tumours of both the decapitated and undecapitated controls.

TABLE I. (Tomatoes).

	TREATMENT	No. of inoculations	TUMOURS					HOST PLANTS							
			No. May 24.	No. June 20.	No. end of exper.	Tot. fresh weight g.	Tot. dry weight g.	Tot. height m. May 24.	Tot. height m. June 9.	Tot. height m. June 20.	Tot. height m. July 23.	Tot. height m. end of exper.	Tot. weight of stems kg.	Tot. weight of roots kg.	Size of stomata μ
Decapitated	Acenaphtene alone	—	—	—	—	—	—	2.38	5.42	8.70	24.14	38.79	3.58	0.67	6.1±1.14
	Acenaphtene + inoculation	80	7	17	16	11.82	1.59	2.37	5.58	8.30	24.37	36.75	3.13	1.07	13.5±1.17
	Control I. uninoculated	—	—	—	—	—	—	3.36	7.90	11.66	24.88	38.29	3.09	0.68	7.6±0.86
	Control II. inoculated	80	65	76	63	59.59	7.58	3.73	7.08	11.80	24.50	38.38	3.38	0.98	8.2±0.97
Undecapitated	Control III. inoculated	80	76	67	72	58.77	7.99	7.92	11.71	12.88	25.97	41.02	3.25	0.60	7.3±1.05
	Control IV. uninoculated	—	—	—	—	—	—	7.56	9.87	11.02	26.56	39.79	3.34	0.69	7.4±0.86

2. Podophyllin.

a) *Ricinus.* 67 days after the inoculations the tumours had developed sufficiently to present a wide enough range of sizes (see Fig. 4), from small to very large, to put to the test the degree and range of activity of the drug. Table II. shows the sum of the largest diameters of the podophyllin tumours and of the lanolin controls, before, and 11 days after the first treatment, in categories according to size. These categories were established to facilitate the distribution of the tumours of the podophyllin and control groups into two equal lots and were based upon the average of the largest diameters of the tumours. 9 categories were set up, numbered from 1 to 9, the respectively largest average diameters of the tumours being in the same order: 35, 30, 25, 18, 16, 13, 10, 7, and 5 mms. (see Table II.)

TABLE II. *(Ricinus).*

Added diameters (mm) of tumours ranged in different size-categories.

No. of size category and av. diameter mm.	Podophyllin			Lanolin		
	No. of tumours	Tot. of diam. before treatment	Tot. of diam. after treatment	No. of tumours	Tot. of diam. before treatment	Tot. of diam. after treatment
1. (35)	3	106	93	2	69	71
2. (30)	2	62	48	3	88	90
3. (25)	3	72	56	2	53	51
4. (18)	3	49	33	3	59	56
5. (16)	4	67	32	5	77	80
6. (13)	9	123	54	9	111	135
7. (10)	8	77	40	9	93	108
8. (7)	5	35	10	5	35	47
9. (5)	3	15	10	3	15	21
Totals	40	606	381	41	600	659

After the second treatment (18 days after the first) the tumour reduction induced by podophyllin was still more striking. Even the largest had considerably shrunk and blackened, and the smaller had shrivelled up or peeled off in layers and could be detached easily. At the same time the larger lanolin tumours remained unchanged and the smaller ones continued even to grow. The untreated control tumours behaved similarly. After the third treatment the destruction of the podophyllin tumours became so rapid that many of them shrivelled to negligible size almost over night. Figures 5 and 6 show some representative plants after the first, and after the third treatment. The plant on the left of these photographs is the one represented on Figure 4 before any treatment. It was noticed after the second treatment, however, that podophyllin had also damaged the healthy tissues, and that around the tumours of many plants the stems had blackened and become woody. It must be further pointed out that the oncolithic influence of podophyllin was almost negligible on *Ricinus sanguineus* plants, as compared with the above.

At the end of the experiment the tumours were cut off but, owing to the circumstance that among the podophyllin tumours only the largest ones could be detached unbroken, while the remains of the smaller ones had to be scraped off and fell to pieces, no individual tumour weights can be given. Enough will be said in stating that, while the total weight of the podophyllin tumours was 5.81 gs, the lanolin tumours weighed 38.10 gs.

It is also worth noting that *secondary tumours* were found on two of the podophyllin-treated plants at a great distance from the uppermost inoculation (Fig. 7.). It may be added that, while cultures of the pathogenic organism could be readily obtained from the uppermost tumour of one of these plants, no bacteria could be isolated from the secondary tumour. This comes certainly as near as is compatible with the anatomical and physiological differences between plants and animals, to the metastatic tumours of these last.

b) Tomatoes. There is little to report as regards the oncolithic action of podophyllin on tomato tumours, the attempt having been a failure. It may, however, be worth noting that the drug damaged the healthy tissues of the host plants less than it did those of *Ricinus*.

3. Vitamin K. *Pelargoniums*. The above tomato strain of *B. tumefaciens* was hardly virulent enough for the variety of *Pelargonium* used: out of 220 inoculations only 55 „took" and 55 days after the

inoculations only four tumours had reached the size of 12 mms. diam. The other size categories were 4, 6, 8, 10 mms, 75 % of which ranged between 4 and 8 mms. 34 days after the first, and 25 days after the second treatment the vitamin K tumours seemed, if anything, rather larger than smaller than the lanolin controls. This impression was confirmed at the end of the experiment by the weight of the tumours, which was, for an equal number of tumours, 14.00 gs in the vitamin K series, 12.40 in the lanolin group and 13.20 gs for the untreated tumours. In the above experimental conditions the curative effects of vitamin K on the phytocarcinomata of *Pelargonium* were in consequence nil.

DISCUSSION.

Most of the experimental data, outlined above, seem self-explanatory enough to dispense with a detailed comment. Some real or apparent contradictions and the problems raised thereby, deserve, however, to be thrown into relief.

It may be asked, first of all, whether the inhibition of tumour induction and development by acenaphtene was not rather due to a general poisoning of the host plants — as manifested initially by the drastic stunting of their growth — than to a differential sensitivity of tumour tissue to the emanations of the chemical.

A *point d'appui* for this interpretation can be found in the empirical fact that fast-growing, sappy plants, such as were the controls, respond more readily to inoculations of *Bacterium tumefaciens* and produce larger tumours than stunted, slowly-growing, more woody plants. This difference in sensitivity is, of course, also manifested in response to other oncogenic agents, both physical and chemical (see f. i. HAVAS, 1939, LEVINE, 1942). But if this is true it is, as generally admitted, not less true that the meristematic tumour tissues grow even faster than the host plants. Their greater sensitivity to oncogenic agents, or other *noxia,* can be inferred following the same reasoning. Animal cancer therapy is based, as is known, on the same differential susceptibility of the tumour and host tissues. The circumstance that when the growth-inhibiting action of the drug decreased it was accompanied by an increased production of tumours (see Table I.), seems to corroborate the above interpretation.

Yet it might be asked why the average size of individual tumours in the acenaphtene plants was so much inferior to the size of the

control tumours, and this despite the fact that by the end of the experiment, and even much before, the growth-rate of the acenaphtene plants had overtaken, and even hyper-compensated, the growth rate of the controls. One cannot help thinking that the main reason for this was that the first impact of acenaphtene on the bacteria, when the host tissues were still impregnated with the emanations of the drug, had sufficiently impaired, not only their vitality and their rate of multiplication (as shown by the reduction of „takes"), but also their virulence, their „toxicity", (as shown by the size of the tumours) to exert a durable action. As to the mechanism of this lasting effect, the hypothesis that it might have been the inhibition of the auxin synthesis of the bacteria, is perhaps not too highly speculative. It is based, on the one hand, on the possibility of a change in the synthetic capacity of the cells, similar to that described, f. i., by BEADLE and TATUM (see WHITE 1947) in the mutant of *Neurospora,* and, on the other hand, on the influence of acenaphtene on auxin production and translocation, as shown in higher plants by different growth-effects (HAVAS and FELFÖLDY l. c.), parthenocarpy (HAVAS 1946) and epinasty (HAVAS, unpublished).

Another aspect of the combined actions of acenaphtene and the bacterial infection which deserves to be emphasized, was their synergism, as revealed by their compound effects, in eliciting polyploid structures (see criteria in prev. chapter). The respective size of the stomata of the leaves in the different groups: (acenapthtene, treated and untreated, inoculated and uninoculated), illustrates this fact (Table I.). It must be noted, on the other hand, that the failure in obtaining polyploid shoots or leaves in the decapitated and inoculated control plants was contrary to our expectations as based upon the findings of WINGE, (1927, 1930), KOSTOFF and KENDALL (1932), KRENKE (1933), HAVAS (1942) and many others, according to which the combined actions of wounding and inoculation with *B. tumefaciens* frequently give rise to polyploid shoots especially from tumour tissue. Neither were our expectations fulfilled as regards the combined actions of decapitation and acenaphtene in the uninoculated control plants, this treatment having in other experiments (to be reported later) elicited polyploid chimeras. The circumstance that the combined actions of acenaphtene and the bacterial infection were sufficient to stimulate the emergence of polyploid organs is all the more remarkable, as acenaphtene alone did not give rise to such, and

that it suppressed, as demonstrated above, the development of many tumours from which polyploid shoots might have risen.

Owing to their simpler technique, the podophyllin experiments present no such problems. Yet it is worth noting that while the drug was highly active in the tumours of *Ricinus comm.* (var. *Mauthner*), it had no effect at all on the tumours of the variety of tomato used. There were even indications, as far as the small number of the tumours of *R. sanguineus* permitted to conclude, that these were equally refractory to the oncolithic influence of podophyllin.

As to the lack of response of the bacterial galls of *Pelargonium* to vitamin K, apart from its possible inactivity, the rather woody structure of the abnormally slowly developing tumours might have been one of the causes of their lack of sensitivity. Questions of species specificity, as above, may also have played a role.

Summing up the lessons taught by the above experiments, it may be stressed once more (cf. HAVAS 1937, 1939, 1940, 1942 etc.) that, impossible as it is to formulate a chemical or physical definition of carcinogens or of polyploidogenic agents (see also DUNLAP and WARREN 1946), it is just as impossible to give a generally valid definition of the physical and chemical characters of the oncolithic agents in plants and animals. Yet, in view of the above and the many other previously revealed analogies of response of plants, and animals to polyploidizing, carcinogenic and carcinolithic sensitizers (some of which were reviewed by HAVAS 1939, 1940, 1942 and LEVINE 1945), the existence of a common denominator of these actions can reasonably be assumed.

DUNLAP and WARREN (l. c.) have also pointed out the „striking changes of activity that follow the slightest alteration of structure" w i t h i n the different groups of carcinogens. According to them such changes imply a high degree of specificity in the process of chemical carcinogenesis.

May we add that a similarly high degree of specificity must be a characteristic of the chemism of the hosts, as shown by their finely graduated and often divergent responses to the self-same agents, examples of which were given above. The importance of such e n d o g e n o u s factors cannot be enough emphasized.

SUMMARY.

Examples were given of the oncolithic influence of the polyploidizing agents acenaphtene and podophyllin, on „phytocarcinomata",

induced in tomato and castor oil plants by *Bacterium tumefaciens*. Another polyploidogenic agent, vitamin K, failed to elicit tumour reduction in *Pelargonium zonale*.

Polyploid shoots were observed in tomato plants as a result of the combined actions of acenaphtene and the bacterial infection, while neither acenaphtene alone, nor the inoculations alone gave rise to similar structures.

Species and race-specific responses were observed in the different plants to the administered chemicals, in view of which attention was drawn to the importance of endogenous factors in the reactions observed.

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LITERATURE CITED.

1. BROWN. N. (1939): *Phyopath.* **29.** 221.
2. „ (1939a): *Rept. Proc. 3rd Int. Congr. Microbiol. N. Y.* 548.
3. „ (1942): *Phytopath.* **32.** (I). 25.
4. CZAPEK, F. (1925: *Biochemie der Pflanze.* 3. Aufl. **2.** 426. Fischer.
5. DERMEN, H., BROWN, N. (1940): *Am. J. Cancer.* **38.** 169.
6. „ „ (1940a): *J. Heredity.* **31.** 197.
7. DUNLAP, CH. E., WARREN, SH. (1946): *Cancer Research.* **6.** (9), 453.
8. HAVAS, L. J. (1937): *Nature.* **140.** 191.
9. „ (1937a): *Bull. Assoc. Française Cancer,* **26.** (6) 1.
10. „ (1939): *Nature.* **143.** 789.
11. „ (1940): *Conf. Soc. R. Sci. Méd. et Nat. Bruxelles.* 8 Janv.
12. „ (1942): *Bull. Acad. Roy. Belgique, Cl. Sci. V. S.* **28.** 318.
13. „ (1946): *Nature.* **157.** 620.
14. „ (1948) *Experientia.* **4/2,** 69.
15. „ FELFÖLDY, L. (1947) *Arch. Biol. Hung.* S. II. **17.** 131.
16. ISSEKUTZ, B. (1941): *Gyógyszerek és gyógyítás. Eggenberg, Budapest.* **1.** 171.
17. KING, L. S., SULLIVAN, M. (1947): *Arch. of Pathol.* **43.** 374.
18. KOSTOFF, D. (1938): *Nature.* **142.** 753.
19. „ KENDALL, (1932): *Science. N. S.* **76.** 150.

20. KRENKE, M. P. (1933): *Wundkompensation, Transplatation und Chimaeren bei Pflanzen. Berlin.*
21. LEVINE, M. (1942): *Cold. Spring Harbor Symp.* **10.** 70.
22. „ (1945): *Bot. Rev.* **11.** 145.
23. NYBOM, N., KNUTSON, B. (1947): *Hereditas.* **33.** 58.
24. SOLACOLU, T., CONSTANTINESCO, M. et D. (1939): *C. R. Soc. Biol.* **130.** 1148.
25. SULLIVAN, M., WESCHLER, H. J. (1947): *Science.* **105.** 2730.
26. WHITE, PH. (1947): *Ann. N. Y. Acad.* Sc. **49.** 111.
27. WINGE, O. (1927): *Z. f. Zellforsch.* **6.** 397; (1930) ibid. **10.** 638.

EXPLANATION OF FIGURES ON PLATES III. AND IV.

Fig. 1. Fasciated stems of tomato plants inoculated with *B. tumefaciens* and treated with acenaphtene.
Fig. 2. Adventive roots emerging from stems and tumours of tomato plants inoculated and treated with acenaphtene.
Fig. 3. Shoot with fruit emerging from a tumour of a tomato plant treated as above.
Fig. 4. Range of tumours of *Ricinus* treated with podophyllin.
Fig. 5. Tumours of *Ricinus* after first treatment with podophyllin. The plant on the left is that represented on Fig. 4, before any treatment and has already shed some of the smaller tumours.
Fig. 6. The same plants after the last treatment.
Fig. 7. *Ricinus* plant with secondary tumours on a leaf.

Fig. 1.

Fig. 2.

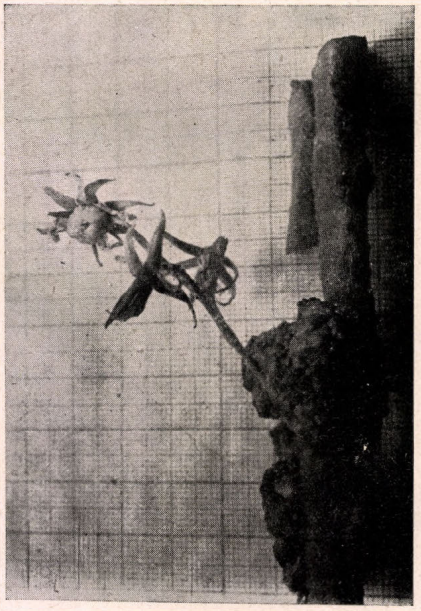

Fig. 3.

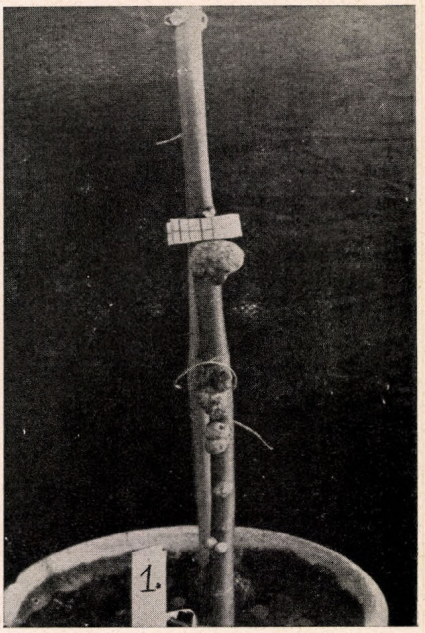

Fig. 4.

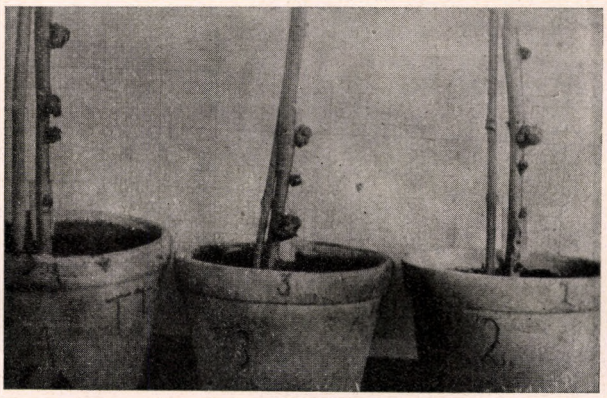

Fig. 5.

Fig. 6.

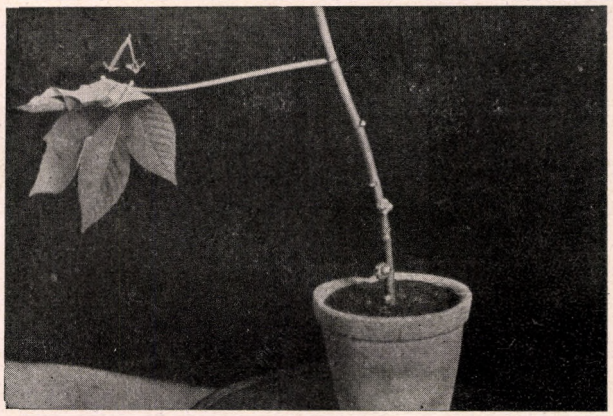

Fig. 7.