Although some of the above preliminary findings are suggestive of hormonal effects, further experiments are in hand to establish how far this claim is justified.

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Colchicine and Acenaphthene as Polyploidizing Agents

FROM the communication by B. R. Nebel[1] in NATURE of August 6 reporting that acenaphthene solution does not induce chromosome doubling in *Tradescantia*, I believe that he is not well acquainted with the method by which I worked. In my first paper[2] I described the method as follows: "Soaking seeds from *Triticum vulgare*, *Tr. monococcum*, and *Secale cereale* in saturated aqueous solution of acenaphthene, with excess of crystals (sublimated particles), during two days and then putting them into Petri dishes, watered with the same solution, we found that the seedlings react morphologically in the same way as they react to colchicine solution" (p. 198). In the treated material polyploid cells and sectors were found. Excess of crystals was necessary (which act in the form of sublimated particles), since saturated solution alone was not sufficient to induce chromosome doubling. (I shall not quote here the further elaborations of the method[3] described in my paper quoted by Nebel, since it was published in NATURE.)

We know now quite well that the active principles[4,5,6] are the sublimated particles, since dry acenaphthene crystals act effectively from a distance in inducing chromosome doubling; therefore I am applying it now by covering the plants or the shoots with reagent tubes (glasses) or cylinders the walls of which are covered with acenaphthene crystals from inside. For some experiments we dissolved the crystals in ether, shook up the solution in the reagent tubes and cylinders, the ether evaporated rapidly and the walls of the tubes and cylinders were left covered with crystals.

Treating branches of *Nicotiana longiflora* with acenaphthene sublimating particles, I produced tetraploid and octaploid shoots from which seeds were produced and further polyploid plants were raised.

Applying colchicine solution I obtained tetraploid plants from *Nicotiana rustica*, *N. tabacum*, *N. glauca*, *N. suaveolens*, *N. megalosiphon*, *N. Velutina*, *N. alata-Sanderæ*, *N. suaveolens-alata*, *Petunia hybrida*, *P. parviflora*, etc., and octaploid in *N. alata-Sanderæ*. Treating *Lactuca* (salad) germinating seeds with colchicine solution, I obtained only polyploid cells, but no polyploid shoots or plants. Tetraploid *Lactuca* plants were obtained, however, by treating germinating seeds with acenaphthene sublimated particles from crystals.

In studying the procedure of the meiosis and mitosis in plants treated with acenaphthene and colchicine[2-7], and inducing polyploid cells, sectors and whole shoots and plants by these two agents in many species and hybrids, I have collected data on the basis of which I can affirm (contrary to Nebel) that acenaphthene interferes with the mitotic and meiotic processes in a way similar to that of colchicine, creating conditions for chromosome doubling. Thus acenaphthene can be successfully applied for inducing polyploidy, as had already been done in *Nicotiana*, *Triticum*, *Lactuca*, etc.

It should be mentioned here that the effect of the acenaphthene is increased with increase in the amount of sublimating particles and in the time of exposure. The quantum of the particles increases with increase of crystal surface and with increase of temperature (within limits). Large amounts of sublimated particles, and also a long time of exposure without interruptions, may injure and even kill plant tissues.

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Production of Growth-Substance by Clover Nodule Bacteria

THIMANN[1], using the standard *Avena* technique, showed that a growth-substance is produced in considerable amount in root nodules. He furthermore claimed that the growth-substance produced is not derived from the meristematic tip of the nodule, but comes directly from the bacterial tissue. He found that the symptoms induced by 3-indole-acetic acid upon roots closely resembled those produced, in Molliard's work, by the action of sterile filtrate of nodule bacteria upon pea roots, and he consequently believed that the bacteria cultivated in laboratory media produce growth-substance in considerable amount.

Using Went's pea test technique[2], I have confirmed Thimann's view that nodule bacteria do produce a good deal of growth-substance in a culture provided with a small amount of tryptophane in the medium. The filtrates of four weeks old cultures of strains of clover nodule bacteria grown in a yeast-water medium containing 0·02 per cent tryptophane were tested against pea shoots prepared according to Went's method. The results of a typical experiment are shown in the accompanying table.

	*p*H	Dilution				
		1/4	1/8	1/16	1/32	1/64
Uninoculated control	8·2	0	0	—	—	—
Strain 2057 in medium without tryptophane	8·4	0	0	0	0	0
Strain 2057	7·9	+	+	+	±	0
Strain 2027	8·0	+	+	+	±	0
Strain 202	7·8	+	+	±	0	0
Urine	–	+	+	±	–	–

+ Positive reaction. 0 No reaction.
± Reaction doubtful. – Not tested.

It appears that strains that are effective in fixing nitrogen in the plant produce in this tryptophane medium very little if any more growth-substance than do the non-beneficial strains that are not