indicates the difference in nature between immature and aged ova as it affects their later development.

In a previous study of follicular oocytes of unmated or pregnant rabbits (*2*), it was found that (i) nuclear maturation as far as first polar body formation occurred when the oocytes were transferred into the Fallopian tubes, (ii) fertilization occurred at the stage of first polar body formation following transfer into the tubes of mated animals, and (iii) the proportion of fertilized ova was increased when the oocytes were cultured for 12 hr before transfer. From the results of the present study, it seems that the maturation of oocytes as a whole is probably more important for their future development than nuclear maturation, because none of the cultured oocytes developed into fetuses, although their nuclear maturation was observed after culture and their fertilization should have occurred in the Fallopian tubes. On the other hand, it may be that culture of oocytes in serum has adverse effects on the cytoplasm, even though nuclear maturation occurs.

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Tumor Induction on *Nicotiana* Species by Use of Coconut Milk and Yeast Extract

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It has been known for some time that coconut milk (*1*) and more recently crown gall tumor extracts (*2*) have a growth-promoting effect on explanted tissues. This paper (*3*) deals with the induction of nongenetic plant tumors on *Nicotiana* species (which otherwise have not been observed to form tumors) by the physiological action of coconut milk and yeast extract. Tumors of different origin but of somewhat similar nature have been reported on *N. alata* (*4*).

Surface sterilized seeds of *N. glauca, N. alata,* and the tumor-forming amphidiploid, *N. glauca-langsdorffii,* were grown on 8 ml of special media (adjusted to *p*H 5.9) in 2½-in. screw-top vials. The basal tobacco medium used in all cases was that of Hildebrandt and Riker (*5*); three supplemental media were made by adding coconut milk (15 percent by volume), malt extract (5 g/lit), and yeast extract (5 g/lit) to this basal medium. Both the malt and the yeast extract were the dried commercial product, and it is possible that different lots may vary in their potency to produce tumors. Autoclaving was done at 15 lb of steam pressure for 20 min. Each treatment consisted of 20 vials, although contamination and lack of germination resulted in fewer than this in some of the final readings.

Table 1. Incidence of tumor formation in sterile culture vials on seedlings of *N. glauca* and *N. alata* on various substrates (planted 24 Oct. 1953; readings taken 1 Dec. 1953).

Medium used	*N. glauca*		*N. alata*	
	Tumors (%)	Non-tumors (%)	Tumors (%)	Non-tumors (%)
Basal (containing 0.1 μg/lit NAA)	0	100*	0	100
Basal plus 15% coconut milk	95	5	25	75
Basal plus 2½% malt extract	0	100	0	100
Basal plus 2½% yeast extract	60	40	33	66
Basal plus coconut milk and malt extract	88	12	66	33
Basal plus coconut milk and yeast extract	20	80	0	100
Basal plus malt extract and yeast extract	58	42	100	0

* Percentage of total uncontaminated vials with sprouting seedlings.

The first experiment was completed using the species *N. glauca* and *N. alata.* It can be observed from the summary in Table 1 that in no instance did tumors develop on either species when the seedlings were germinated and grown on mineral media without supplements or on media containing only malt extract. However, tumors did appear more or less consistently on all seedlings growing on media that contained coconut milk, yeast extract, and combinations containing these two materials with malt extract (Fig. 1). The tumors were white in color, except when malt extract was present in the media; in this case they appeared as a brownish mass. They varied markedly in size from those just barely detectable to those about 10 to 12 mm in diameter. The tumors appeared to arise from the stem base, but the stem layer from which the tumor cells originated is unknown. The roots did not seem to be affected. It was observed that, subsequent to the appearance of the described tumors, other tumors appeared on the larger roots of a few cultures of *N. alata.* These latter tumors appeared to be similar to those described by Tryon (*4*).

Since the basal medium in the first experiment contained 0.1 μg/lit of naphthaleneacetic acid (NAA), a

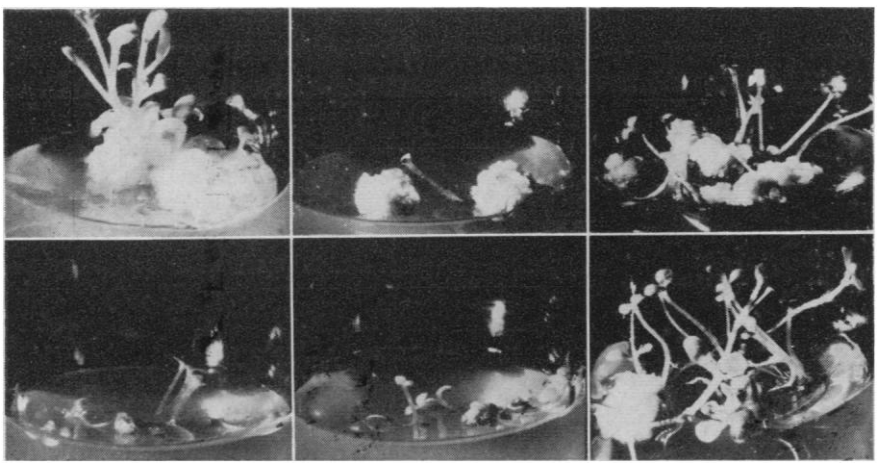

Fig. 1. Tumors formed on *Nicotiana* seedlings growing in sterile culture tubes on various media. Top, left to right: *N. glauca* on coconut, *N. glauca* on yeast extract medium, *N. glauca* on yeast-coconut medium. Bottom: *N. glauca* on malt-coconut medium, *N. alata* on malt-yeast medium, *N. alata* on malt-coconut medium.

second experiment was run using *N. glauca* and two levels of NAA. The results of this experiment indicated that a growth regulator in the medium was not essential for the initiation of the tumors described.

A final experiment was run similar to the first experiment with the exception that the amphidiploid, *N. glauca-langsdorffii,* was substituted for *N. alata.* This amphidiploid possesses a genetic constitution that conditions the plant toward the development of spontaneous tumors under natural environments (*6*). Germination was exceedingly poor (whether by chance or otherwise the amphidiploid did not in any case germinate on any media that contained yeast extract either alone or in combination). The results with *N. glauca* were almost identical to the results of experiment 1; and on the amphidiploid, as was expected, tumors appeared on the seedlings germinated on all media, including most vials of the straight mineral. Unexpected, however, was the decided stimulatory effect of the plant extracts, particularly coconut milk, on tumor development (Fig. 2). These materials advanced both the time of appearance of the tumors, and the degree to which they developed. It should not be inferred that the tumors on the amphidiploid were induced by the plant extracts, since such tumors are initiated solely by genetically controlled mechanisms under natural environments. These results indicate that the plant extracts used not only have a tumor-inducing potential on the normal diploid species but have a stimulatory action as well on the tumor growth of *N. glauca-langsdorffii.*

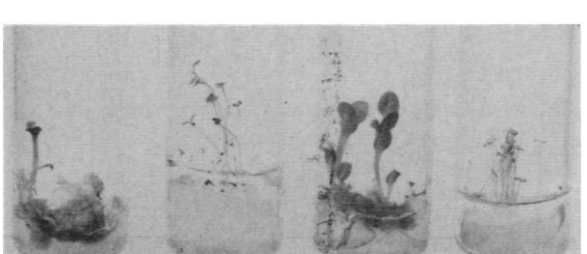

Fig. 2. Tumor-inducing and stimulatory effects of coconut milk on *Nicotiana* seedlings. Left to right: *N. glauca-langsdorffii* on coconut medium, *N. glauca-langsdorffii* on straight mineral medium, *N. glauca* on coconut milk, *N. glauca* on straight mineral medium.

The nature of the tumors induced on *N. glauca* and *N. alata* by coconut milk or yeast extract has not been completely determined, but it is probable that they belong in the same category as those produced by Brown and Gardner using high concentrations of growth regulators (*7*). It is doubtful that the tumor condition would be perpetuated if it were implanted into one of the parental species. These tumors, however, can be maintained on tissue-culture media with the aid of a growth regulator but do not seem to thrive on a hormoneless medium. In this respect they differ from tumors of crown gall or those of genetic origin, both of which are autonomous *in vitro* from the standpoint of hormone synthesis. It has already been pointed out that they likewise differ from Tryon's root tumors on *N. alata.*

The technique outlined here may be useful as a biological assay for isolating the growth-promoting components of coconut milk and yeast extract.

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