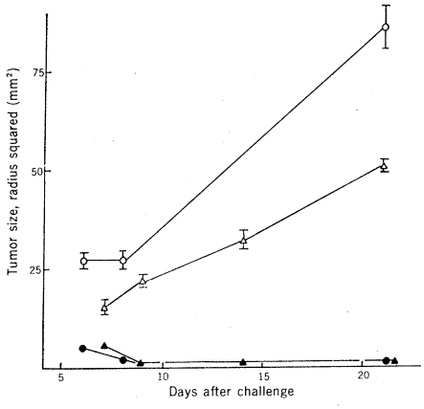

Fig. 1. Intradermal growth of 10^6 line 10 tumor cells in guinea pigs immunized to living BCG alone (△), to living line 1 tumor cells alone (○), or to mixtures of living BCG and living line 10 tumor cells (●, ▲). The results of two experiments are shown. In experiment 1, five guinea pigs were immunized intradermally with BCG alone, and three guinea pigs were immunized with a mixture of BCG and line 10. Each of these eight animals was challenged with line 10 tumor cells 35 days later. In experiment 2, three guinea pigs were immunized intradermally with line 1 tumor cells alone and five guinea pigs were immunized with a mixture of BCG and line 10. Each of these was challenged with line 10 tumor cells 31 days after immunization. The difference at day 21 between tumor nodule size in animals immunized with BCG alone, or immunized with line 1 alone compared to animals immunized with a mixture of BCG and line 10 is significant, at $P = .01$, as determined by t-test.

BCG occurs, and there is no progressive tumor growth. Animals immunized in this way are capable of suppressing the intradermal growth of tumor cells in a challenge inoculum containing ten times the lethal dose.

Strain-2 male guinea pigs (Sewell-Wright, inbred) were obtained from the breeding colony at the National Institutes of Health. The induction of primary hepatomas in the guinea pig by feeding the water-soluble carcinogen, diethylnitrosamine (*2*), and the antigenic and biologic characteristics of the transplantable hepatomas derived from the primary tumors have been described (*3*). We used ascites tumor line 10. This tumor, a poorly differentiated hepatocarcinoma, grows progressively after inoculation of 10^5 tumor cells intradermally. The tumor regularly metastasizes to lymph nodes draining the site of tumor injection. Animals injected intradermally with cells of this tumor line usually die about 60 days after injection. The Phipps strain of BCG was obtained from the Trudeau Mycobacterial Collection at a concentration of 1.2×10^8 bacteria/ml in Middlebrook 7H9 broth with Tween (*4*). The guinea pigs were immunized by intradermal inoculation of 6×10^6 bacteria mixed with 1.5×10^6 tumor cells. From 31 to 35 days after immunization, animals were challenged intradermally with 10^6 tumor cells.

The results of representative experiments presented in Fig. 1 and Table 1 can be summarized as follows. (i) All animals immunized with BCG and line 10 tumor cells completely suppressed the growth of line 10 cells. Protected animals have remained tumor free from the initiation of the experiment to the present, a period of 3 months. (ii) Previous immunization with BCG alone did not impair the growth of line 10 cells. (iii) Animals immunized with ascites line 1 did not suppress the growth of line 10 cells. (iv) Animals immunized with BCG and line 10 showed delayed cutaneous hypersensitivity reactions to line 10 cells alone and suppressed line 10 tumor growth. (v) Animals immunized with BCG and line 10 did not show delayed cutaneous hypersensitivity reactions to line 1 cells or suppress line 1 tumor growth. Line 1 is a transplantable ascites tumor induced by diethylnitrosamine in strain-2 guinea pigs. We have been unable to immunize against line 10 cell growth by conventional methods. Our results suggest that it may be possible to produce strong tumor immunity to weakly antigenic tumors by injecting the living tumor cells together with living BCG.

The paucity of reports of the use of complete Freund's adjuvant to induce specific cell-mediated tumor immunity suggests a lack of success with this method. Perhaps the labile tumor specific transplantation antigens are destroyed during emulsification. The procedure outlined does not require emulsification and evidently preserves antigens necessary for induction of specific tumor immunity.

There is already clinical evidence that BCG may be of value in the control of human cancer (*5*, *6*). One approach has been to inject BCG directly into intradermal metastases (*6*). Another approach would be to attempt the induction or augmentation of systemic tumor immunity by the inoculation of autologous living tumor cells mixed with BCG. Our work indicates that for the development of optimal systemic tumor immunity, direct contact between tumor cells and BCG is required (*7*).

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7 August 1970; revised 25 September 1970 ■

Hydroxy-L-proline- and 2,2′-Dipyridyl-Induced Phenovariations in the Liverwort Nowellia curvifolia

Abstract. *Two antagonists of "normal" proline-hydroxyproline-protein synthesis, 2,2′-dipyridyl and hydroxy-L-proline, induced the same kind of phenovariation in* Nowellia curvifolia *(Dicks) Mitt. (Cephaloziaceae) as they do in* Scapania nemorosa *(Scapaniaceae). This finding supports a hypothesized cardinal role for proline-hydroxyproline-protein in modulating aspects of morphogenesis and phylogeny in the leafy liverworts.*

Hydroxyproline and 2,2′-dipyridyl, compounds that interfere with the "normal" synthesis of hydroxyproline-protein, can induce phylogenetically and systematically significant changes in symmetry, branching pattern, and leaf morphology in the liverwort *Scapania nemorosa* (*1*, *2*). Because the pheno-

variation induced in *S. nemorosa* by these compounds causes the gametophyte to produce structures and assume forms which are at once phenocopies of other taxa and reminiscent of possible earlier stages in the evolution of leafy gametophytes, it was suggested that hydroxyproline-protein might play a cardinal role in modulating the aforementioned aspects of morphology during both the ontogeny and phylogeny of leafy liverworts (*3*).

If this hypothesis is correct, then the procedures which led to phenovariation in *Scapania* (Scapaniaceae) could be expected to induce similar changes in other genera belonging to other families. I now report the first evidence supporting the hypothesized general role of hydroxyproline-protein in influencing liverwort morphogenesis and phylogeny.

As a first test of the hypothesis, *Nowellia curvifolia* was used because of its morphological similarities to and systematic differences from *S. nemorosa*. As does *Scapania, Nowellia* lacks ventral leaves and its branching is infrequent. Branches, when they do occur, are typically ventral in origin. On the other hand, *Nowellia* is a member of the Cephaloziaceae, a family which most bryologists recognize as distinct from the Scapaniaceae. And, if the admittedly speculative phyletic schemes of Schuster are correct (*4*), the Cephaloziaceae is derived from a line that diverged early and evolved separately from the one leading to the Scapaniaceae.

The procedures used to culture and treat the plants and the composition of the basal culture medium have been described (*1, 5*). The 15 cultures used in this experiment were initiated with about 500 cells per flask from a suspension of gemmae derived from a single stock culture of *Nowellia curvifolia* (Dicks) Mitt. They were incubated under continuous illumination from a combination of cool white fluorescent and incandescent lamps at 1880 to 2150 lux and at a temperature of 19° ± 2°C. The plants were treated when they reached the juvenile stage (*6*). Five replicate cultures received either 2,2′-dipyridyl (dipyridyl) to give a working concentration of 4 μg/ml, or hydroxy-L-proline (hydroxyproline) to give a working concentration of 5 μg/ml. The five controls received the basal culture medium. After 5 weeks of incubation in the presence of the proline antagonists, cultures were harvested and the gametophytes were carefully inspected under a dissecting microscope to assess morphological effects. The outline drawings of leaves used for Fig. 1 were made by tracing photographs.

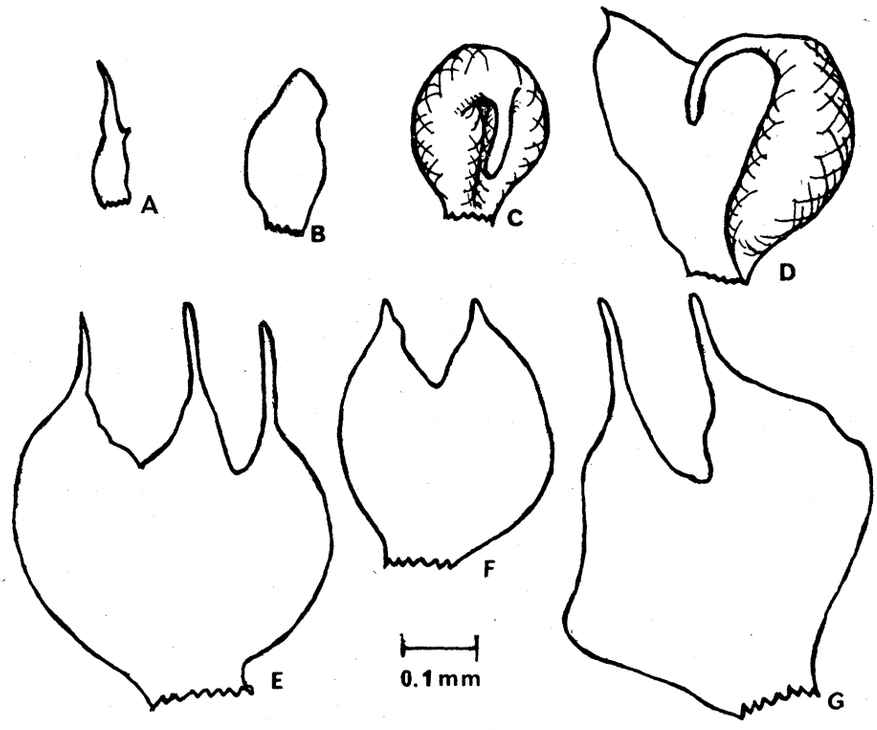

Fig. 1. Leaves formed on phenovariants of *Nowellia curvifolia*. (A) Subulate, ventral leaf. (B) Ligulate, ventral leaf. (C) Saccate, ventral leaf. (D) Concave-saccate lateral leaf characteristic of adult *Nowellia*. This type of leaf also developed in a ventral position on occasion. (E) Plane, trilobed lateral leaf. (F) Plane, bilobed ventral leaf. (G) Plane, bilobed lateral leaf. Leaves E, F, and G constituted a three-leaf sequence.

Both dipyridyl and hydroxyproline induced the same kinds of phenovariations in *Nowellia* as they did in *Scapania*. Although all replicates contained phenovariants, the extent of phenovariation per plant and number of phenovariants per culture showed a range similar to that reported for *Scapania,* and probably for the same reasons (*1*). The most conspicuous effects were development of ventral leaves and increased branching.

The ventral leaves varied considerably in form from subulate through ligulate to saccate, monolobed leaves as well as plane bifid and concave-saccate bilobed leaves (Fig. 1). In the few instances when plane, bifid ventral leaves occurred in conjunction with plane, bifid, lateral leaves or concave-saccate, ventral leaves with concave-saccate, lateral leaves, perfect triradial symmetry was approached. Triradial symmetry is rare in leafy liverworts and has been considered as either the primitive condition or a derived condition by different bryologists (*4, 7*). Moreover, the formation of ventral leaves places these phenovariants outside the range of characteristics that presently circumscribe the genus *Nowellia*.

Increased branching was mostly endogenous and ventral in origin but terminal exogenous branching also occurred. Branching in the controls was rare and only of the terminal exogenous type. The analogs therefore induced a change in the branching pattern from a restricted type to a freer one. Branching patterns are also important in phylogenetic and systematic considerations (*3, 4, 7*).

Lateral leaves did not exhibit a wide range in basic form in this experiment. They were either plane bifid or concave-saccate. A few three- and four-lobed leaves were found in experimental cultures, however, which suggests that *Nowellia,* like *Scapania,* may be capable of wider phenovariations.

Although designed to test one hypothesis, the results of this simple experiment with *N. curvifolia* provide support for three hypotheses formulated earlier from work with *S. nemorosa* (*1–3*). (i) Because their morphogenetic influence is indistinguishable and the most likely common physiological effect of the two diverse com-

pounds is to interfere with normal synthesis of hydroxyproline-protein, the results presented herein further support the hypothesized general role for hydroxyproline-protein in liverwort morphogenesis. (ii) Because the action of both antagonists would logically result in the altered synthesis of hydroxyproline-protein, this experiment further supports the hypothesis that the development of three rows of leaves, rather than two, and unrestricted branching are somehow causally related to a limited formation or limited hydroxylation of hydroxyproline-protein, or both. (iii) Because changes in morphology were clearly not permanent (phenovariants reverted to their characteristic morphology) it is assumed that *Nowellia* like *Scapania* has retained, rather than obtained, the genetic capacity to form ventral leaves and to branch freely. This is considered strong support of the hypothesis that triradial symmetry with free branching is the primitive condition in the leafy liverworts and dorsiventral symmetry with restricted branching is derived (*3, 4, 7*).

Of course, even though the foregoing hypotheses are supported by work with representatives of two well-separated families of the Jungermanniales, similar evidence from experiments with representative species of several other families must be obtained before any firm conclusion concerning the role of hydroxyproline-protein in the phylogeny and morphogenesis of the leafy liverworts can be reached.

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17 August 1970

Genetic Variation of Cholesterol Ester Content in Mouse Adrenals

Abstract. *Total cholesterol concentrations in muscle, liver, and plasma do not differ significantly between adult male C57BL/10J and DBA/2J mice. In the adrenal glands of these two strains, of their hybrids, and of AC mice, concentrations of free cholesterol vary by 5 percent. Adrenals from C57 mice, however, contain six times as much esterified cholesterol as adrenals from AC or DBA mice. The intermediate concentrations of cholesterol esters in F_1 hybrids suggest that the difference in this measure is inherited additively. The finding of variation in adrenal cholesterol within a species is useful for the further study of the role of cholesterol in steroidogenesis by means of genetic analysis.*

Current theories on steroid hormone production in the adrenal gland ascribe a special role to the cholesterol ester fraction. During short-term stimulation, cholesterol from the ester fraction provides substrate for the formation of the corticosteroid secreted by the gland (*1*). After prolonged stress the adrenals are depleted of the normally present lipid droplets (*2*). It was recently found that one of the early events in the mechanism of action of adrenocorticotropic hormone (ACTH) is the hydrolysis of cholesterol esters (*3*). The extent to which this pool of cholesterol contributes to the dynamics of hormone production has, however, not been studied sufficiently.

The two inbred strains of mice C57BL/10J and DBA/2J have been observed to respond differently to stress in terms of plasma corticosterone concentrations (*4*). In an attempt to find biochemical mechanisms for this apparent genetic difference in stress response, we studied the involvement of adrenal cholesterol. We report here large differences in cholesterol ester concentrations in adrenal glands of different strains of mice.

The AC mice and colonies of the pure-bred strains C57BL/10J and DBA/2J and their F_1 hybrids were maintained in our laboratory. The colonies were started from breeders purchased from the Jackson Memorial Laboratory, Bar Harbor, Maine. Litters were weaned at about 24 days after birth and housed in groups of three to eight. Breeding pans always contained two females and one male. The F_1C hybrids were derived from crossing a C57 female with a DBA male; the F_1D hybrids were derived from crossing a DBA female with a C57 male. Experiments were performed exclusively on adult male mice (10 to 20 weeks old). In experiments in which more than one type of mouse was used, the tissues were coded and analyzed without knowledge of their strain classification.

The mice were killed by decapitation. Blood was collected in small, dry, heparinized beakers. Centrifugation in a Beckman microfuge for 2 minutes yielded 0.1 to 0.3 ml of plasma. Small samples of liver and muscle (from the hind leg) were collected in one experiment. Adrenal glands were removed and freed from adhering fat. The tissues were kept ice cold and moist with isotonic NaCl. Before weighing, they were blotted briefly with filter paper.

Tracer amounts of [26-^{14}C]cholesterol and [7α-^{3}H]cholesteryl stearate were added to the tissue (100 mg of muscle or liver, 0.10 ml of plasma, or one pair of adrenals), which was then extracted twice with 3 ml of a mixture of acetone, ethanol, and ethyl ether (4:4:1, by volume) (*5*). A glass rod was used to break up the tissue in a centrifuge tube. The combined extract was evaporated with N_2. The residue was dissolved in 3 ml of a mixture of

Table 1. Weights of pairs of adrenal glands, body weights, and ratios of adrenals to body weights of pure strains of mice and hybrids (C57 mice crossed with DBA mice). For further details see text. Values shown are the means of the means obtained in n different experiments (number in parentheses) and the standard errors of the means. Because no statistically significant (at $P \leq .05$) differences were found between the two reciprocal hybrids in any of the three parameters, the values for the hybrids are pooled for the purpose of this presentation.

Strain	Adrenal glands (mg)	Body (g)	Ratio (mg/100 g)
C57BL/10J	3.0 ± 0.04 (7)	28 ± 0.6 (15)	11 ± 0.2 (4)
DBA/2J	3.1 ± 0.07 (10)	26 ± 0.3 (16)	12 ± 0.6 (5)
F_1 Hybrids	3.4 ± 0.09 (9)	29 ± 1.0 (7)	12 ± 0.3 (6)
AC	2.7 ± 0.04 (3)	21 ± 1.3 (3)	13 ± 0.9 (3)