was distinctly anomalous and suggestive of additional entirely different components (*7*).

The two hundred available *R. insulatus* were "milked" of secretion by the technique used for similar purposes in other species (*5*). In contrast to the secretion of other quinone-producing millipedes, which show a single carbonyl absorption band in the 6.0 μ region, the crude secretion of *R. insulatus*, in methylene chloride, showed two carbonyl absorptions of comparable intensity in its infrared spectrum (5.91 and 6.04 μ). This suggested the presence—in addition to quinone(s)—of a conjugated aldehyde or ketone. Sublimation of the crude secretion (25°C/10 mm-Hg) gave a yellow crystalline sublimate (mp 69°–70°C). This material was identified as 2-methyl-1,4-quinone by comparison of its infrared spectrum with that of an authentic sample (*8*) (6.04, 7.8, 9.2, 11.1, and 12.4 μ), and comparison of gas chromatograms (20 percent Carbowax; 187°C).

The unknown component absorbing at 5.91 μ was isolated by chromatography on activity-III alumina (Merck, 71707) with pentane as eluent. This carbonyl component, which was eluted before the 2-methyl-1,4-quinone, showed characteristic infrared absorption at 3.48, 3.55, 3.70, 5.91, 6.13, 6.9, 8.9, 9.1, and 10.3 μ. The proton magnetic resonance spectrum of this material suggested the partial structure $-CH_2-CH=CH-CHO$ [peaks at 0(doublet)(1),2–4(multiplet)(2), and 7.7(2)τ] with 17 additional protons on saturated carbon. The ultraviolet spectrum, in ethyl alcohol, showed absorption maxima at 223 and 315 mμ, with extinction coefficients of 10,000 and 19, respectively. This spectrum is consistent with a mono-substituted α, β-unsaturated aldehyde.

This aldehyde was identified as *trans*-2-dodecenal by comparison with an independently synthesized authentic sample. Reduction of 2-dodecenoic acid (*9*) with lithium aluminum hydride gave *trans*-2-dodecenol (90 percent) with infrared maxima at 3.1, 3.5, 6.1, 6.9, and 10.3 μ. Oxidation of the alcohol with chromium trioxide-pyridine (*10*) gave *trans*-2-dodecenal (31 percent) (bp 73°–74°C at 0.5 mm-Hg; reported (*11*) 125°–128°C at 10 mm-Hg). The infrared spectra of the synthetic *trans*-2-dodecenal and that of the natural product from the millipede were superposable, and their gas chromatographic retention times were identical (5 percent silicone SE-30; 185°C). A mixture of the natural and synthetic material gave a single symmetrical peak on the same column. Finally, the semicarbazone of the synthetic material melted at 159°–160°C, the reported (*11*) values being 160°C and 165.5°–166°C, and the semicarbazone of the natural product melted at 160°–161°C; the melting point of the mixture was undepressed.

In order to estimate the ratio of aldehyde to quinone in the crude secretion, the infrared spectra of known mixtures of *trans*-2-dodecenal and 2-methyl-1,4-quinone were examined. The known mixture, which showed all the absorptions of the crude secretion except a small peak at 11.3 μ, established the ratio of aldehyde to quinone as 2.5:1. This ratio was confirmed by a comparison of the weights of chromatographic fractions.

Data on the protective action of the secretion will be published elsewhere as part of a comparative study of the repellent effectiveness of a variety of natural products. Suffice it to say, *R. insulatus* is virtually invulnerable to attack by aggressive predators such as ants (*Formica exsectoides* Forel) and grasshopper mice [*Onychomys torridus* (Coues)]. Surprisingly, several of the millipedes were eaten in quick succession by an ornate box turtle [*Terrapene ornata* (Agassiz)], which betrayed no ill effects either during or after the meal.

2-Methyl-1,4-quinone has been obtained not only from other millipedes (*4, 5*) but from other arthropods as well (*1*). *trans*-2-Dodecenal has been found prevoiusly in plants [citrus (*12*), ginger (*13*), and carrot (*14*) families], but although other α,β-unsaturated aldehydes are known from animal secretions (*1, 15*), this appears to be the first reported occurrence of *trans*-2-dodecenal in the animal kingdom.

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Dictyotene Stage of Meiosis in Mosses

Abstract. *The dictyotene stage of first meiotic prophase, characterized by an elongation of the paired chromosomes after diplotene, and known to occur during the growth period of the oocytes of many animals, has a morphologically analogous but relatively short-lived counterpart in the first meiotic prophase of representatives from three moss families.*

A meiotic stage morphologically analogous to the dictyotene stage which occurs during prophase I of oogenesis in many animals has been discovered in three mosses, namely, *Hypnum circinale* Hook., *Brachythecium frigidum* (C. Meull) Besch., and *Claopodium crispifolium* (Hook.) R. & C. The fact that these species represent three families of mosses suggests that the dictyotene stage may be of wider occurrence in this group of plants. This is the first observation of the dictyotene stage in plants and indicates that this meiotic phase is not unique to the animal kingdom. Furthermore, the dictyotene stage, which undoubtedly has some fundamental significance in the meiotic sequence in mosses, may have significance in the evolution and phylogeny of this plant group.

In the three species studied, the stages of first meiotic prophase appear similar except for minor variations. A description of prophase I in *Hypnum circinale* can be taken as representative.

When the archesporial layer of the capsule of *H. circinale* has ceased divid-

ing mitotically, the individual cells become spherical, assuming the form of premeiotic spore mother cells.

The first prophase stage, leptotene, is characterized by an acentric nucleus containing very fine, tightly packed, unpaired chromosomes. Synapsis follows. The chromosomes assume a typical pachytene morphology and the nucleus migrates to the center of the cell. As pachytene proceeds, the chromosomes shorten and thicken, and a classical diplotene phase appears as the repulsion of homologues becomes evident. Diplotene is the most commonly found prophase stage in *H. circinale* and may persist a week or more in nature. In rare spore mother cells all six bivalents can be discerned, but usually only one or two are separated from the main chromosomal mass (Fig. 1*a*). The smallest bivalent often becomes heteropycnotic and highly condensed. At no time can the nuclear membrane be distinguished when these cells are stained with aceto-orcein. In addition, the nucleoli are extremely small and difficult to observe.

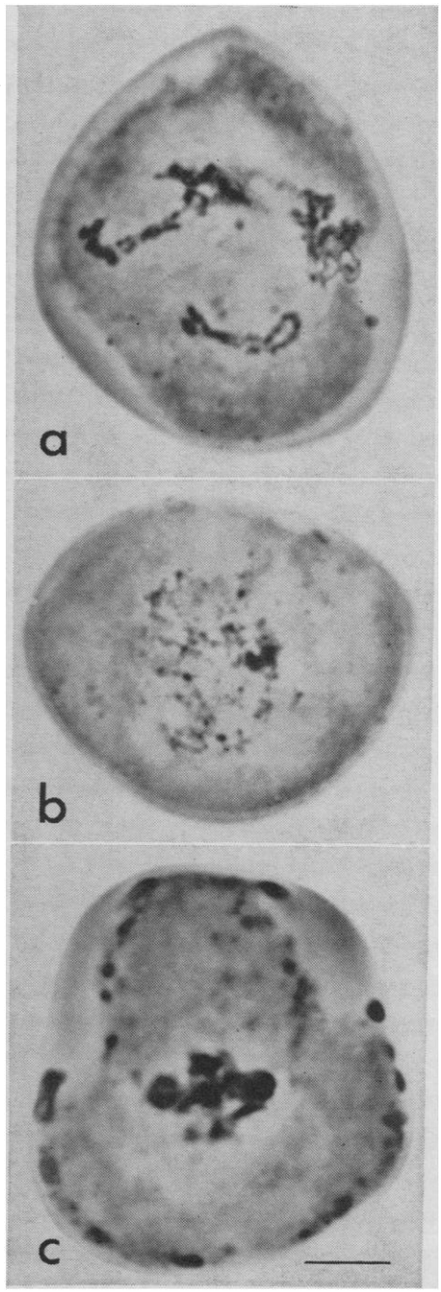

Fig. 1. Spore mother cells of *H. circinale* in late stages of prophase I of meiosis: (*a*) diplotene, (*b*) dictyotene, (*c*) diakinesis. Line indicates 5 μ.

At the end of diplotene the spore mother cells enter the dictyotene stage. The chromosomes gradually lengthen and lose their discreteness, at which time the nuclear contents assume an interphase-like appearance (Fig. 1*b*). Utilizing phase contrast, one can observe fine chromatin threads and what appear to be chiasmata. These observations indicate, perhaps, that the basic diplotene structure is maintained. These dictyotene nuclei also contain at least one deeply staining chromatic body.

Up to dictyotene, meiotic events in the mother cells of any one capsule are almost synchronous. From this stage on, however, the synchrony is lost. The cells may remain in dictyotene for 2 to 3 days, then proceed to the next stage in a random order by a rapid condensation of the bivalents. Initially the chromatin becomes extremely diffuse, then dense and contracted. Finally the chromosomes can be distinguished as a clumped diakinesis (Fig. 1*c*). A characteristic metaphase I configuration with six bivalents follows and meiosis continues in a classical manner. The complete meiotic sequence may extend over a period of 2 to 3 weeks.

The species dealt with here were unlike many mosses in that the prophase chromosomes could be satisfactorily stained with aceto-orcein. However, observations of prophase I were hampered if the collected material was subjected to increased temperature between the time of collecting and examining. No capsules with spore mother cells in diplotene were observed in material of *H. circinale* kept at room temperature for 24 hours. All were either in prediplotene stages or dictyotene and later stages. Apparently the cells that were in diplotene had proceeded to dictyotene and those that were at the end of pachytene experienced an extremely transitory diplotene during the 24-hour storage.

Few studies have been reported on the development of meiotic prophase stages in mosses. In an attempt to study the meiotic prophase of *Hedwigia ciliata* (Hedw.) Br. & Sch. and some members of the Grimmiaceae, Vaarama (*1*, *2*) found that poor stainability of the spore mother cells at prophase I rendered detailed examination impossible. He noted (*1*) that prophase stages later than pachytene have been rarely observed, which suggests that these stages are of short duration. Later, however, the same author (*3*) succeeded in studying prophase I of *Pleurozium schreberi* (Brid.) Mitt. and presented the only published figure of true diplotene in mosses known to me. Nevertheless, he did not describe or depict the dictyotene stage and indicated that a clumped diakinesis directly followed diplotene. No other detailed studies of meiotic prophase in mosses exist.

It is noteworthy that many workers in moss cytology have suggested bringing material into the laboratory to speed maturation. Mosses sensitive to moderate heat stress, as in *H. circinale*, might never be observed in diplotene if such treatment were applied. It is possible that if diplotene were not observed, then the dictyotene stage would be interpreted as a leptotene or zygotene stage.

As far as I know, the occurrence of the dictyotene stage has not been reported in any other group of plants. A stage of postsynaptic elongation of chromosomes is known to exist in the developing asci of many ascomycetes (*4*), but this stage occurs before diplotene and is a characteristic pachytene.

During meiotic prophase in most male animals and in higher plants diakinesis directly follows diplotene by a shortening and thickening of the paired chromosomes. However, in the oocytes of many female animals, the dictyotene stage intervenes. In mammals, chromosomes of the dictyotene stage are usually diffuse, superficially resembling those of interphase (*5*, *6*). In amphibians and some insects, on the other hand, the paired chromosomes take on the appearance of the well-known "lampbrush" stage (*7*). Although the outward appearance of the dictyotene nuclei varies considerably in different animal groups, the basic diplotene morphology of the chromosomes is maintained (*7*, *8*). Dictyotene generally persists until ovulation, when the meiotic sequence is resumed. During the intervening period, which in man may be from 12 to 45 years, the oocytes grow and accumulate nutrients which are utilized by the future embryo (*5*).

In contrast to the case in animals, the dictyotene stage observed in mosses is not accompanied by an appreciable growth of the spore mother cells, nor does it persist for a relatively long time in the meiotic sequence. Obviously too little information is available, at present, to state the functional significance of this stage in the meiotic events of mosses.

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Inhibition of Bacterial Growth by Drugs of the Morphine Series

Abstract. *The growth of* Escherichia coli *is reversibly inhibited by drugs of the morphine series. The order of inhibitory effectiveness for the drugs tested was levallorphan > levorphanol > dextrorphan > nalorphine > morphine. The synthetic analgesic, levorphanol, was studied in greater detail. Its effectiveness was found to be strongly dependent on the* p*H of the medium. Raising the* p*H of the medium provides a higher concentration of the neutral free base which is thought to diffuse across cell membranes more readily. However, considerations other than the rate of entry of drug into the cells must be of importance since an already established growth inhibition is promptly reversed upon lowering the* p*H of the medium. Two mutants of* Escherichia coli *with altered sensitivity to levorphanol were isolated.*

An investigation of the effects of narcotic analgesic drugs on single cells was initiated in the hope of elucidating the biochemical action of these compounds. Recent studies on the effects of morphine and related compounds on human cells in tissue culture (*1*) stimulated us to investigate the action of these compounds on bacteria. It has been reported that unicellular organisms are unaffected by high concentrations of morphine in the growth medium (*2*), and this finding was confirmed by us with cultures of *Escherichia coli*. However, when closely related drugs, known to be more toxic than morphine to animals, as well as to cells in culture, were examined, bacterial growth was found to be inhibited. The present report deals with the inhibition of bacterial growth, particularly that of *E. coli*, by levorphanol tartrate and other drugs closely related to morphine in structure. The isolation of two mutants of this organism, which differ in their sensitivity to levorphanol, is also described.

The addition of levorphanol tartrate (*3*) at a concentration of $3 \times 10^{-3}M$ to *E. coli*, strain W, growing logarithmically in a minimal medium, resulted in complete inhibition of growth (Fig. 1) (*4*). The fact that the culture showed no decline in absorbancy at a wavelength of 490 mμ in the presence of the drug indicates that there was no detectable cell lysis. Viable cell counts made by spreading suitable dilutions of aliquots of the cultures on nutrient agar plates showed that the growth inhibition was reversed when the drug was removed from the medium, and that little or no cell death had occurred. Identical results were obtained when this strain was grown in enriched media, such as nutrient or peptone broth, provided the *p*H was kept near neutrality by the addition of a buffer. Under the same conditions morphine had no effect at its limit of solubility; nalorphine showed inhibition at a concentration of about $10^{-2}M$; while dextrorphan, the enantiomorph of levorphanol, was 20 to 30 percent less effective than levorphanol. Levallorphan, the *N*-allyl analogue of levorphanol, was somewhat more inhibitory than levorphanol. Thus, for the drugs tested, the general order of effectiveness in inhibiting bacterial growth parallels their order of toxicity in intact animals (*5*) and in human cells in tissue culture. Efforts to demonstrate antagonistic effects of the *N*-allyl derivatives against their parent compounds have so far been unsuccessful.

The *p*H of the medium has a marked effect on growth inhibition by levorphanol. Below *p*H 6 no inhibition was demonstrable. The effective dose is 3 to $4 \times 10^{-3}M$ when the *p*H of the medium is near neutrality, and inhibition decreases steadily as the *p*H is raised. At *p*H 8.5, the highest *p*H at which good growth of *E. coli* was obtained, multiplication was inhibited completely by $1 \times 10^{-3}M$ levorphanol, and some inhibition of growth was observed at concentrations as low as $1 \times 10^{-4}M$.

In a variety of systems molecules cross cell membranes more readily in the neutral than in the charged state. Raising the *p*H could, therefore, provide a higher concentration of unprotonated base for diffusion into the cells. On the basis of this hypothesis one would predict that once an inhibitory dose had entered the cells it would be effective regardless of the *p*H of the medium. This prediction is not borne out. It can be seen from Fig. 2 that a culture incubated at *p*H 8.2 with an inhibitory dose of levorphanol long enough to be inhibited, and presumably long enough for the drug to have reached its site of action, resumed growth promptly when the *p*H was lowered to 6.5. Thus the simple hypothesis that the effectiveness of the

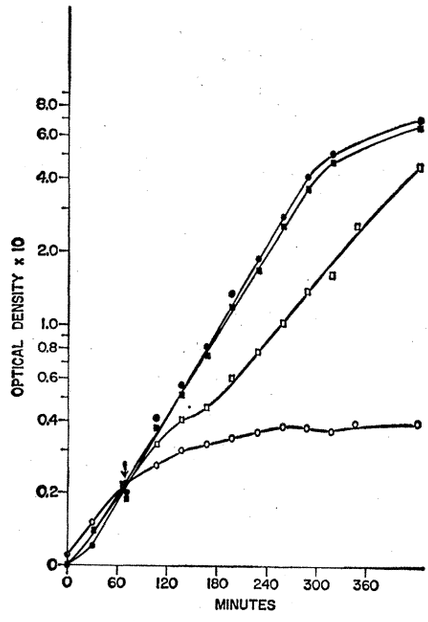

Fig. 1. Inhibition of *E. coli*, strain W and mutant S-6 by levorphanol at *p*H 6.7. Strain W and mutant strain S-6, which exhibits some resistance to the action of levorphanol at neutral *p*H, were grown in minimal medium (*8*) with 0.5 percent sodium lactate as the carbon source. Levorphanol tartrate was added at the time indicated by the arrow. Closed circles, strain W control; closed squares, mutant S-6 control; open circles, strain W + $2.7 \times 10^{-3}M$ levorphanol tartrate; open squares, mutant S-6 + $2.7 \times 10^{-3}M$ levorphanol tartrate. Growth was followed by optical density measurements at 490 mμ in a Lumetron colorimeter.