The data on isolated secretion products for both wild-type and *msg* mutant *T. confusum* are compared in Table 1. Although both secreted 2-methyl- and 2-ethyl-1,4-benzoquinone, the amount obtained from the wild type was approximately 20 times greater in each case. From neither strain was 2-methoxy-1,4-benzoquinone detected, and only the wild type gave the corresponding alkylhydroquinones.

Both strains yielded the same amount of colorless (and relatively odorless) oil by this procedure. Although this substance was not completely identified, some information on its structure was obtained. This oil has the molecular formula $C_{14}H_{26}O$ (molecular weight 210 by mass spectrometry), showed a carbonyl absorption in the infrared at 1710 cm^{-1}, and $—CH_3$ (δ 1.1) and $=CH_2$ (δ 5.1) absorption in its nuclear resonance absorption. Since there was no aldehydic proton absorption, this compound appears to be an acyclic ketone with a terminal, nonconjugate (very weak ultraviolet absorption at 285 mμ) vinyl group.

The black material in the gland reservoirs of mutants is a high-molecular-weight, polymeric material. It appears to consist of polymerized quinones and may have arisen because the inhibitor of hydroquinone oxidation (*5*) is absent. The quinones thus may be formed prematurely and polymerize in the reservoir. This also is consistent with the much decreased amount of alkylquinone and absence of hydroquinone in the secretion of *msg*.

There has been much speculation about the role of the odorous secretions in *Tribolium*. They may have played a defensive role when *T. castaneum* and *T. confusum* occupied a different habitat; but as Roth (*1*) has emphasized, today *Tribolium* encounters few predators in the flour it infests, and quinones appear to be ineffective in warding off mites which may be the flour beetles' chief predators in their present habitat.

Van Wyk *et al.* (*9*) find that *T. confusum* is generally attracted to flour containing storage fungi or bacteria isolated from the beetles themselves, but as the population increases "the population of storage fungi decreases almost to the vanishing point, presumably because the quinones, secreted by the beetles, are toxic to the fungi." These authors believe that one of the functions of the malodorous secretion is to keep the food material relatively free of microorganisms which, if they were allowed to grow without restraint, would compete directly with, or make the substrate unsuitable for, the insect.

It would seem that Van Wyk *et al.* (*9*) are correct in ascribing such a function to the beetles' malodorous secretions. We observed that the medium in crowded cultures of normal beetles remained particulate, and did not become moldy; but in crowded cultures of the mutant, the medium developed a grey-green mold and became cakey. Also, the population of the mutant appeared to increase more rapidly than that of the wild type.

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4 August 1965

Hydra: Induction of Supernumerary Heads by Isolated Neurosecretory Granules

Abstract. *Neurosecretory granules of* Hydra littoralis *were isolated by differential centrifugation. Excised midsegments of hydra that were exposed to the fraction containing neurosecretory granules developed additional heads at any site, as they regenerated. Hence neurosecretory granules may contain a factor that regulates or stimulates growth in normal and regenerating hydra.*

The nervous system of hydra appears to be necessary for regeneration because exposure to any one of several inhibitory neuropharmacological agents evokes abnormal regeneration or inhibits regeneration (*1*). At a fine structural level, some nerve cells in hydra contain dense, membrane-bounded granules, 1000 to 1200 Å in diameter, in the perikaryon especially in close relationship to the Golgi apparatus and in the neurites and their endings (*2*). After transection, these granules, presumably neurosecretory in nature, accumulate in the nerve terminations and are released into the intercellular spaces at the regenerating site (*3*). These observations suggest that a growth-stimulating or form-regulating factor may be located in the neurosecretory granules. In order to investigate this possibility further, neurosecretory granules were isolated by differential ultracentrifugation, and

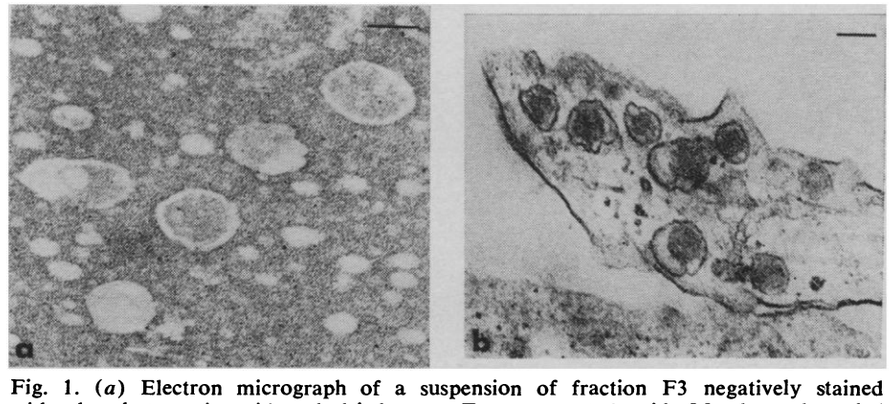

Fig. 1. (*a*) Electron micrograph of a suspension of fraction F3 negatively stained with phosphotungstic acid and dried on a Formvar-coated grid. Membrane-bounded granules, 1000 to 1200 Å in diameter, are present in addition to smaller particles. (*b*) Electron micrograph of a thin section of a neurosecretory cell neurite containing dense, membrane-bounded granules. Bars represent 0.1 μ.

their effect on regenerating hydra was assayed.

Cultures of *Hydra littoralis* were grown in the laboratory by the methods of Loomis and Lenhoff (*4*). For each fractionation procedure, carried out at 4°C, approximately 10,000 hydra, which had been fed 24 hours previously, were homogenized in 15 ml of 0.4*M* sucrose. The homogenate was centrifuged first at 3000*g* for 10 minutes to sediment the first pellet or fraction (F1). The F1 supernatant (S1), on centrifugation at 10,000*g* for 10 minutes, yielded a second pellet (F2). The resulting supernatant (S2) was then centrifuged at 35,000*g* for 60 minutes, and a final pellet (F3) and supernatant (S3) were produced. This fractionation procedure was selected after several trials at different speeds and durations of centrifugation to yield, by morphological standards, adequate separation of morphologically recognizable elements.

Some of the pellets were suspended in 2 percent phosphotungstic acid (*5*), and droplets of this preparation were placed on Formvar-coated copper grids and allowed to dry prior to examination with an electron microscope. Fraction F3 contained numerous, large (1000 to 1200 Å), moderately dense membrane-bounded granules (Fig. 1*a*). In addition, the matrix of the pellet between the granules contained small particles of several sizes and densities and a few membranous elements (Fig. 1*a*). The isolated granules were quite similar to neurosecretory granules observed *in situ* in hydra neurons (Fig. 1*b*). A few similar structures were also present in fraction F2, but this fraction was composed mainly of fragmented mitochondria and other membranous elements. The first fraction (F1) contained intact and partially disrupted cells, nuclei, and nematocysts.

For bioassay, the pellets were resuspended in 10 ml of 0.4*M* sucrose. These suspensions could be stored at −20°C for several months without loss of activity. Assays were performed on segments of the body column removed from the middle of the stomach region. Intact hydra or transected hydra were not used for assay because the assay material was less effective on them than it was on isolated midsegments. Hydra culture-water (tap water containing tetrasodium ethylenediamine tetraacetate, 0.05 g/liter; $CaCl_2$, 0.05 g/liter; and $NaHCO_3$, 0.10 g/liter) was added to the suspensions containing fractions F1, F2, and F3 to produce a final concentration of 1 to 100 by volume. The final supernatant (S3) was diluted five times with water. The midsegments of normal hydra were placed in these solutions for 4 hours immediately after removal of the base and head. They were then transferred to fresh culture water for the remainder of the regenerative period. Controls consisted of midsections of hydra allowed to regenerate in culture water without added homogenates. Regenerates were observed after 3 days, and abnormal forms were noted. Regenerates having supernumerary heads (hypostome and tentacles) or tentacles outside the oral ring were counted. Hydra having an excessive number of tentacles within a single head, forked tentacles, or extra bases were not included because these forms occurred frequently during normal regeneration.

Table 1. Effect of isolated fractions of hydra on regenerating midsegments.

Experiment	Total midsegments (No.)	Abnormal regenerates	
		No.	Percentage
Control	315	14	4.4
F1	220	9	4.1
F2	203	14	6.9
F3	272	68	25.0
S3	244	8	3.2

The results of the assay experiments appear in Table 1. Fraction F3 contained a substance capable of inducing abnormal head formation in 25 percent of the isolated midsegments. Supernumerary heads did not form in the remaining animals which regenerated normally. Some regenerates subjected to material in F3 are compared with a control regenerate in Fig. 2. In the normal situation, a single head forms at the distal end of the cut portion, while a base develops proximally (Fig. 2*a*). After treatment with the solution containing the material in F3, however, two heads frequently appear distally (Fig. 2*b*); an additional head may develop anywhere along the body column (Fig 2*c*); or a head sometimes forms proximally where the base would be (Fig. 2*d*) suggesting the absence of polarity. The results obtained with midsegments of hydra placed in solutions containing material from F1, F2, or S3 are similar to those obtained in control animals (Table 1). The active material in F3 was not

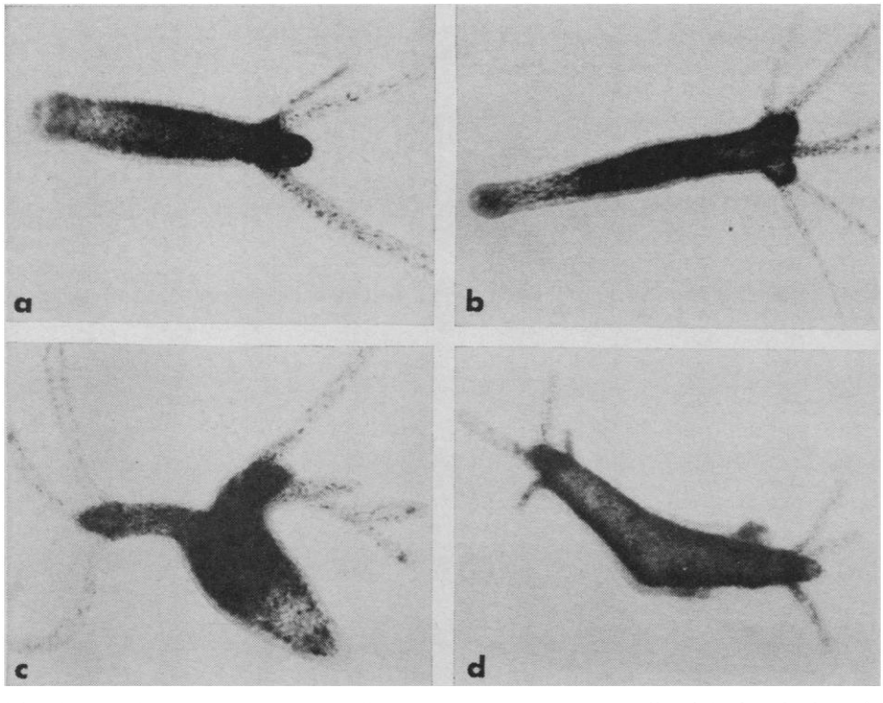

Fig. 2. A midsegment of hydra allowed to regenerate normally (*a*) for 3 days is compared with three others exposed to a fraction (F3) containing neurosecretory granules (*b–d*). The normal regenerate (*a*) possesses a distal head (hypostome and tentacles) and proximal base while those exposed to F3 are abnormal, having two distal heads (*b*), an additional head protruding from the body (*c*), and heads protruding both proximally and distally (*d*).

destroyed when heated at 100°C for 10 minutes, and it was dialyzable.

These results are in agreement with those of Lesh and Burnett (*6*), who obtained a substance from boiled homogenates of hydra capable of inverting polarity. These workers suggested that a growth substance is produced in the hypostome and extends in a gradient from head to base, controlling cell division and head formation along the body column.

These results substantiate my earlier suggestion (*3*) that a growth-stimulating or form-regulating factor is present in the neurosecretory granules. It appears that at least one function of this material is stimulation of head formation because, during regeneration, release of neurosecretory granules occurs at the site where a head is to appear (*3*) and because, in the present experiments, exogenous administration of isolated granules induced formation of supernumerary heads. Thus, neurosecretory granules containing form-regulating substances, when transported to specific sites by neurites, may be responsible for maintenance of form in normal hydra and for the acquisition of form in regenerating hydra.

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16 July 1965

Soluble Proteins of a Melanoma and Normal Skin from the Swordtail, Platyfish, and Their Hybrids

Abstract. *Acrylamide gel electrophoresis has been used to make comparisons of the distribution of soluble protein and the activity of esterase in skin and melanoma extracts of the swordtail, the platyfish, and their hybrids. There is a striking difference between the extracts of normal skin and tumor tissue derived from the same cytological elements. Several prominent protein bands are found only in the tumor tissue. Their origin and function are unknown.*

In the laboratory, under confined conditions, *Xiphophorus helleri* (the swordtail) and *X. maculatus* (the platyfish) will interbreed, and the resulting hybrid is fertile. This interspecies hybridization has received considerable scientific attention (*1*), owing to the fact that the interaction of the genes of the platyfish, which lead to the production of macromelanophore spotting, with certain genes in the swordtail results in the production of a malignant melanoma in the first filial and in subsequent generations.

With the introduction of the techniques of disc electrophoresis in acrylamide gels by Ornstein and Davis (*2*), detailed comparisons of electrophoretic protein patterns with high resolution became possible.

As part of a larger study of the effects of hybridization upon the electrophoretic pattern of extractable proteins (*3*), detailed comparisons were made between the extractable proteins of the normal pigment cell and those obtained from the melanoma.

The purpose of the experiments reported here was to determine whether or not the electrophoretic pattern of the tumor-tissue protein differed significantly from that obtained from the normal cells.

The technique of acrylamide-gel disc electrophoresis used in our observations was that described by Ornstein and Davis (*2*) with the exception of minor modifications.

The fish were raised in our laboratory from inbred stocks originally obtained from the Genetics Laboratory of the New York Zoological Society in the American Museum of Natural History.

Soluble skin proteins were obtained from four groups of fish: (i) the green swordtail (*X. helleri*), inbred 30 generations. In the figures this group will be indicated by (S × S); (ii) the platyfish (*X. maculatus*) inbred 20 generations (P × P); (iii) the swordtail–platyfish first generation hybrid resulting from a cross of the two species above (S × P); (iv) the swordtail–platyfish hybrid backcrossed to the swordtail [(S × P) × S]. This cross is tumor bearing.

The swordtail skin was free of macromelanophores, since these cells do not normally occur in these fish. The platyfish and hybrid skins, used as a source of proteins, were pigmented. Nonmacromelanophore-bearing skin from the backcross served as a control source of skin proteins, since it lacked macromelanophore pigmentation but had the same genotype as the tumor tissue.

The fish were immobilized by placing them on ice. The tissues were removed and ground in a Dounce-type microhomogenizer at 5°C, in sufficient 0.25*M* sucrose to give a final concentration of 10 percent. After grinding, the tissue extract was cleared by centrifugation; the clear supernatant was collected and diluted with sucrose (0.25*M*) to bring the protein concentration in each homogenate to approximately 50 μg per gel tube. The protein concentration was estimated by the method of Lowry (*4*). Serums were obtained by heart puncture. Tissue and serums were applied to the acrylamide gel in 0.25*M* sucrose (*5*).

Variations were observed in the protein electrophoresis patterns among the two species and their hybrids (Fig. 1). These pattern differences were consistently obtained and emphasize the utility of disc electrophoresis in the examination of closely related species (*6*).

All the normal skin extracts showed a relatively simple electrophoresis pattern. In the case of the tumor extract, however, it is apparent that there are a great many more protein bands in the tumor tissue than in normal epi-

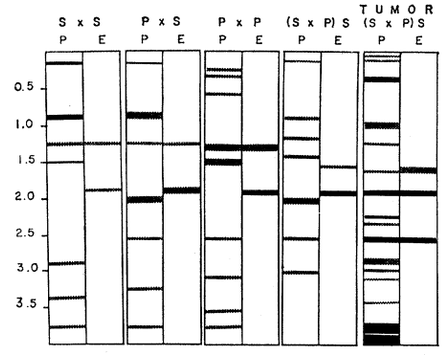

Fig. 1. Soluble proteins of skin and melanoma. S × S, swordtail; P × S, platyfish–swordtail F_1; P × P, platyfish; (S × P)S is F_1 backcrossed to swordtail; *P*, protein; *E*, soluble esterase; ordinate, centimeters.