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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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-

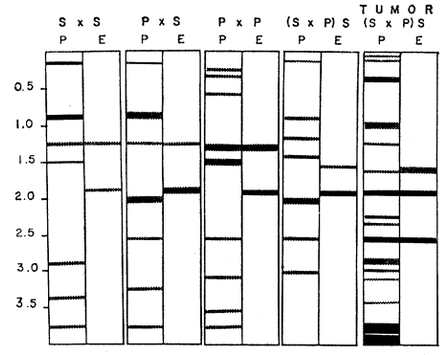

Fig. 1. Soluble proteins of skin and melanoma. S × S, swordtail; P × S, platyfish–swordtail F_1; P × P, platyfish; (S × P)S is F_1 backcrossed to swordtail; *P*, protein; *E*, soluble esterase; ordinate, centimeters.

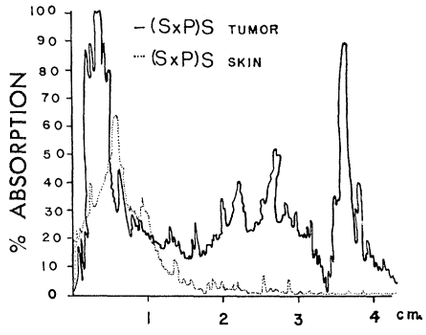

Fig. 2. Densiometric comparison of the soluble proteins of (S × P)S skin and melanoma.

thelial tissue (Fig. 2). The striking difference lies in the changes which have occurred in the relative abundance of the most rapidly migrating proteins and particularly in the occurrence of a very prominent band between 3.8 and 4.0 cm, which appears in the tumor extracts. As can be seen from Fig. 1, very faint bands appear in the 3.8- to 4.0-cm region in the platyfish, swordtail, and hybrid, while in the tumor tissue it is estimated that these bands alone make up 25 percent of the total extractable protein. The function of these protein

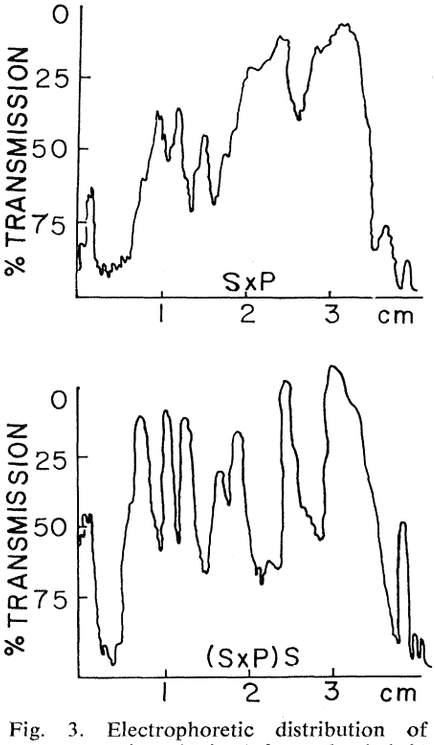

Fig. 3. Electrophoretic distribution of serum proteins obtained from the hybrid (S × P)S. The curve represents a densiometric tracing.

bands is unknown. Analyses so far indicate that these bands have no esterase activity. However, there is in the tumor a new esterase band, migrating about 2.5 cm from the origin.

The possibility existed that these fast-running protein bands represented a considerable incorporation of plasma protein by the tumor tissue. Evidence that such a mechanism exists in several mouse tumors has been presented (*7*). However, the electrophoretic pattern of the backcrossed serum (Fig. 3) shows only a very faint band in the gel in a position corresponding to the tumor protein. It seems more likely to us that the appearance of a small amount of this protein in the serum in this case represents a leakage of the protein into the blood from the tumor, rather than a concentration of the protein from the blood by the tumor.

The changes in the pattern of extractable protein from the pigmented tumor cell, when compared with its normal counterpart, are very striking. That a significant portion of the total protein of the tumor cell runs ahead of the corresponding serum-albumin peak suggests that this protein is not identical with any major component in the serum. Since it is not represented by any similar protein in the normal pigment cell, it is possible that this protein represents a unique product of the tumor. The physiological function of this protein and its relationship to the etiology of malignancy in these cells are not known.

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Differential-Approach Tendencies Produced by Injection of RNA from Trained Rats

Abstract. *Two groups of rats were trained in a Skinner box to approach the food cup when a discriminative stimulus (click or blinking light) was presented. Ribonucleic acid was extracted from the brains of these two groups of rats and injected into two groups of untrained rats. The untrained two groups then manifested a significant tendency (as compared with one another) to react differently to the two stimuli. On the average, the response appeared to be specific to the stimulus employed during training.*

When RNA was extracted from the brains of rats trained to approach a food cup and was injected intraperitoneally into untrained rats, the rats so injected made significantly more approaches to the food cup than did controls (rats injected with RNA from brains of untrained rats) (*1*). We now present evidence that this effect is specific rather than general. Our new experiment is similar to our earlier one, except that we used two experimental groups, each trained to respond to a different discriminative stimulus.

Initially, 16 male Sprague-Dawley rats, aged 50 to 60 days, weighing 220 to 240 g, received magazine training in a standard Grason-Stadler Skinner box; that is, they were trained to approach the food cup when a discriminative stimulus was presented. For eight rats, the discriminative stimulus was the distinct click (*1*). For the other eight rats, the discriminative stimulus was a blinking light. The latter stimulus was produced by blinking the 10-watt house light (within the Skinner box) three times in succession, the three blinks taking a total of approximately 1 second.

For the click-trained rats, magazine-training was accomplished in the same fashion as in our earlier study (*1*).

The blinking light proved to be a somewhat more difficult stimulus to establish as a signal, and accordingly, minor modifications in the magazine-training procedure were made. After early training, the rats did not respond to the blinking light but they did run to the cup upon hearing the slight noise produced by the pellet's dropping into the cup. As training progressed, on more and more trials