liter, but only a fraction is chemically active when this value is corrected to infinite dilution and ion association is taken into consideration.

In the fresh water, the serum concentration of urea is sustained at approximately 132 mmole/liter. Elasmobranchs require at least this concentration of urea for normal cardiac function (*7, 8*). The concentration of total ions is 404.3 mmole/liter, 83 percent of the ion concentration of the serum of marine elasmobranchs; the ionic strength, μ, is estimated to be .2790. Under these conditions, the active concentrations of calcium ions increase to the extent that precipitation of calcium phosphate and depression of neuromuscular irritability can be avoided only if the gill membranes and the kidneys lower the calcium concentration in the body fluids. This level appears in the fresh-water shark to be approximately 3.0 mmole/liter of total and 1.8 mmole/liter of ultrafilterable calcium. The most abundant ions in Rio San Juan and other bodies of fresh water are Ca^{++} and HCO_3^{--}, but the total ion concentration is very low (5.1 mmole/liter), compared with 404.3 mmole/liter in serum. Here, the gills expend energy obtained from intracellular glycolysis to pump ions against concentration and electropotential gradients.

The teleost fish that is found in the same geographic area as the Nicaraguan shark has an entirely different composition of the serum; μ equals 0.15, total ions constitute 266.6 mmole/liter, and the total calcium is only 2.5 mmole/liter. Uremia is absent (Table 1). The blood is less concentrated, more viscous, better aerated, and homeostasis is achieved presumably by dynamic mechanisms using the functions of bone cells and bone tissue as a buffer (*7*). Teleosts are ionically independent and highly advanced, compared with the elasmobranchs; they evolved bone for finer regulation of ionic composition of the blood. The apatite mineral in bone is metabolized by means of a two-way process of resorption and formation, and constitutes the storage depot of a closed cycle or servosystem for utilizing hydronium, sodium, calcium, phosphate, and other ions. In addition to gills, kidneys, and bone, integument functions as an organ of ionic homeostasis. It serves as a semipermeable membrane in lamprey (which have gills but no calcium deposits) and in certain adult amphibians (which possess true bone but no gills and yet retain urea). In elasmobranchs and teleosts, the body covering is waterproof, and facilitates osmoregulation. Thus, the interactions and combinations of organs used by vertebrates for water balance and mineral homeostasis are numerous and highly complex. Physiologic experiments are necessary to measure the capacity of the bull sharks for adaptation. A tagging project would be of great interest to determine the limits of their range, reproductive cycle, species specificity, and ascertain whether they readapt to marine habitat.

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Continuous Culture of a Melanotic Cell Line from the Golden Hamster

Abstract. A melanotic cell line derived from a malignant melanoma of the golden hamster has retained its ability to produce melanin in vitro. This cell line provides an opportunity for studying the synthesis of melanin under various controlled conditions. The modal chromosome number in this cell line is 68.

Shortly after the advent of cell culture, Burrows reported his attempts at growing human malignant melanoma in vitro (*1*). Subsequently, short-term cultures of both animal and human melanoma cells have been described (*2*). For example, Wellings *et al.* (*3*) apparently grew human malignant melanoma cells in vitro for at least 4 weeks. Similarly, cultures of melanoma cells from fish, mice, and hamsters have been described, and have been used for histochemical studies. Rosenberg *et al.* (*4, 5*) cultured both amelanotic and melanotic malignant melanoma cells from tumors which originated in the hamster colony maintained by Fortner (*6, 7*). Rosenberg *et al.* (*4, 5*) reported that the amelanotic tumor was more invasive and grew faster in vivo, an observation which had been previously made by Fortner. Unique differences in the sites of metastases were noted (*4*). They also described the morphological characteristics of monolayer cultures, and stated that there was little growth after the first transfer (*5*).

Table 1. Chromosome numbers in malignant melanoma cells of golden hamster grown in vitro.

Chromosome numbers	Cells counted on sampling dates (1962)	
	12 April	18 April
42–49	3	
50		1
52		1
53	1	
54		1
57	3	3
58	2	1
59	2	3
60	3	
61	2	
62	3	
63	2	3
64	2	4
65	5	9
66	6	11
67	7	13
68*	12	21
69	2	17
70	1	8
71	1	4
72	1	
73	1	1
Total	60	100

* Mode.

A series of melanotic and amelanotic malignant melanomas which developed in aged golden hamsters has been described by J. G. Fortner (*6*). We have successfully cultured several of the amelanotic melanoma tumors in vitro, but we found that either the pigmented tumors did not grow as permanent cell lines or else only nonpigmented cells survived. As a result of a screening program in our laboratory in which various malignant cells are grown in a variety of media with various protein supplements, a melanotic hamster cell line which has retained its ability to

produce melanin has been successfully grown in vitro.

The present culture (RPMI No. 1846) was derived from a spontaneous melanotic tumor which had been carried for 41 animal passages. It was transplanted into several noninbred hamsters in our laboratory in June 1961. On 2 February 1962 biopsy material from these transplants was minced, treated with trypsin, and cultured. The suspension of cells and cell clumps was cultured under perforated cellophane sheets in T-15 flasks. Successful growth was attained in only one flask, which contained RPMI medium No. 651 supplemented with 1 percent fetal calf serum. The original outgrowth was subcultured 33 days later and at 6-day intervals thereafter. The subcultures have been grown in a variety of media supplemented with 20 percent calf serum. All of the subcultures contained some cells with pigment granules. Cells of transfer generations 4, 5, and 7 produced coal-black tumors when reinjected back into hamsters. Cultures containing lactalbumin hydrolysate in the media became very black and the cells were heavily pigmented. These cultured cells with heavier pigmentation did not grow as fast as those in other media.

The chromosome constitution of the melanotic melanoma is shown in Table 1. The melanoma was examined on 2 April and 18 April 1962. On both days the modal chromosome number was found to be 68, with a wide distribution around the mode (range 42 to 73). This contrasts with the sharp mode of 44 chromosomes counted in cultured normal cells of the golden hamster (*8*). In addition, the mode of 68 chromosomes in the melanotic melanoma differs from that described for an amelanotic tumor and a pituitary carcinoma of the golden hamster (*8*).

Karyotyping was performed on 22 plates from cells with a chromosome number ranging from 64 to 71. No unusual marker chromosomes were observed. In all of the plates, 3 or 4 X chromosomes were easily identifiable, and 43 to 49 chromosomes belonged to groups A, B, and C (normal 30 or 31), 9 or 10 to group D (normal 8), 3 to 5 to group E (normal 2), and 3 to 5 to group F (normal 2). It appears that the hyperploidy of this particular cell line is due to an excessive number of chromosomes in all of the groups described for normal cells of the golden hamster (*8*).

This melanotic cell line derived from a malignant melanoma of the golden hamster has continued to produce melanin in vitro. Whether or not it will continue to produce pigment permanently cannot be determined at this time. Nevertheless, this cell line will produce melanin for a prolonged period in vitro and, therefore, should provide a unique opportunity for studying melanin synthesis (*9*).

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Factors Causing Seasonal Forms in Ascia monuste (Lepidoptera)

Abstract. Light effects will produce the seasonal forms of *Ascia monuste* L. but they are not yet proved to be the only causative agent. The long-day form is melanic; the short-day form is white. Only females exhibit the effect. The capacity to form melanic females is most frequent in the Florida population and is probably genetically determined.

Increased recognition is being given to photoperiod as a factor inducing diapause and determining environmental forms in insects (*1*). Experiments with the European butterfly *Araschnia levana* L., for example, are interpreted as indicating that day length is responsible for the striking difference between the spring and summer forms. (*2*).

Before selecting one lepidopteron for experiment, five species with suspected seasonal forms were raised under the two conditions of 16 hours light alternating with 8 hours dark (condition 1, 16L/8D) and 8 hours light alternating with 16 hours dark (condition 2, 8L/16D) in each 24-hour period. The five were *Precis zonalis* Felder, *Precis lavinia* Cr., *Phyciodes phaon* Edw. (Nymphalidae), *Eurema daira* Latreille, and *Ascia monuste* L. (Pieridae). The last was chosen for a further experiment since it gave the clearest phenotypic difference and was easiest to breed. It can be raised from egg to adult in less than a month on young cabbage or other Cruciferae (*3*).

The dimorphism of the Great Southern White butterfly, *Ascia monuste* L., has been attributed to environment (*4*). This species is unusual in that the female alone displays a conspicuous dimorphism. One form is white with narrow black borders on the wings, and the other, form *phileta*, is more or less completely suffused with melanic pigment. Intermediates occur.

Five white females of *Ascia* were collected in December 1960, at the Florida State Board of Health Entomological Research Center at Vero Beach. Eggs were obtained by enclosure of females in lamp chimneys over the food plant *Batis maritima* L. under electric lights. Larvae were raised on young cauliflower plants, since *Batis* is restricted to the coastal salt marshes far from the Archbold Station where the rearing was carried out.

The two light regimes were used. Room temperature was maintained at 80°F. The experiment was performed in the temperature-light control room at the Archbold Biological Station in Lake Placid, Florida. Since only one room was available, a special light-tight box, 13 by 49 inches by 17 inches high (inside dimensions), was constructed for 8L/16D conditions and placed in the chamber. It was equipped with a single fluorescent tube (General Electric F40T124500), baffles, and a fan to give a steady air exchange without admitting light. The room was lighted by a bank of fluorescent tubes containing 15 cool white General Electric F96T12CW and five daylight General Electric F96T12D fluorescent tubes.

Eggs from each female were kept separate and distributed seven to a rearing container. The containers were arranged in pairs housing progeny from the same parent. One of each pair was subjected to 8 hours of light and 16 hours of dark, and the other was subjected to 16 hours of light and 8 hours of dark. Eight pairs ($8 \times 14 = 112$ ova, or 56 under each light condition) were used. Offspring from each of the five females were used in at least one of the pairs.

The result was unequivocal. Of the