develop clinical evidence of ochronosis, a condition characterized by a striking dark brown-black pigmentation of the cartilages, tendons, and sclerae (*2*).

The pigment(s) thus formed have been broadly termed "melanins" (*3*), although no recorded attention appears to have been directed specifically to their nature or properties. However, unpublished observations (*4*) on the solubility characteristics of pigmentary material isolated by differential ethanol centrifugation from the urines of a family segregating for alcaptonuria (*5*) tend to suggest that alcaptonuric-ochronotic pigmentation may result from the selective deposition of one or more compounds having properties somewhat different from those usually attributed to the melanins resulting from the enzymic oxidation of, for example, *o*-diphenol derivatives (*6*). The present study was undertaken, accordingly, as a first approach to the chemical nature of the pigment(s) formed in alcaptanuric ochronosis.

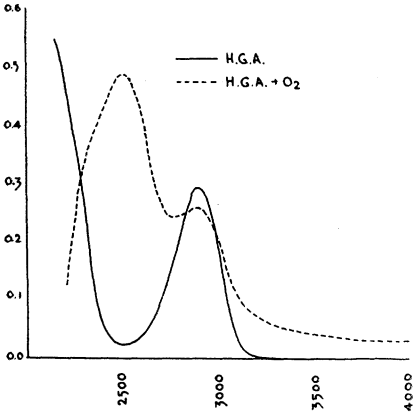

Fig. 1. Ultraviolet absorption spectra of pure homogentisic acid after partial interaction with molecular oxygen. The ordinate is optical density, and the abscissa is wavelength in angstrom units.

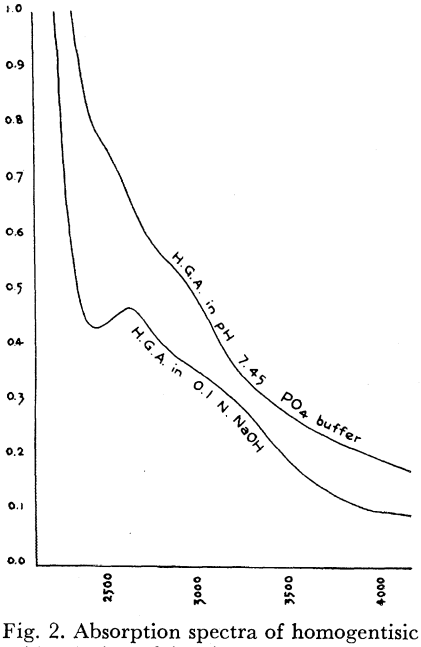

Fig. 2. Absorption spectra of homogentisic acid solutions following prolonged interaction with molecular oxygen. The ordinate is optical density, and the abscissa is wavelength in angstrom units.

Solutions of synthetic homogentisic acid [mp, 146° to 148°C; $\lambda_{max.}$, 2900 A (log ε, 3.58)] (*7*) were prepared in water (*p*H 4.8), phosphate buffers (*p*H 7.4 and 8.0), and 0.1*N* NaOH. Ultraviolet and visible absorption spectra were obtained at room temperature (25°C) with a model 14PM Cary recording spectrophotometer (*8*). Spectra were determined of freshly prepared solutions, of those which had been permitted to stand in air, and of solutions through which both oxygen and air had been passed for prolonged periods of time.

Homogentisic acid showed no absorption in the visible range in water or phosphate buffers, but it showed characteristic absorption at 2900 A, as has been previously reported (*9*). Freshly prepared solutions, however, failed to show absorption peaks other than at 2900 A. Following interaction with molecular oxygen, a second peak gradually developed at 2500 A which coincided with that reported for 1,4-benzoquinone-2-acetic acid, prepared by chromic acid oxidation of homogentisic acid (Fig. 1). The absorption curve obtained by subtracting the spectrum of pure homogentisic acid from the spectrum observed shortly after interaction of homogentisic acid with oxygen, moreover, was found to be identical with that of benzoquinoneacetic acid, thus indicating the presence of an intermediate mixture of both the hydroquinone and quinone derivatives in homogentisic acid oxidation.

Continued atmospheric oxidation resulted in the complete disappearance of the 2900-A peak, concomitant with progressive increase in the intensity of the 2500-A maximum. Additional exposure to molecular oxygen led to the appearance of an entirely altered absorption spectrum, quite unlike that of homogentisic acid in NaOH, that of benzoquinone-acetic acid in water or phosphate buffers, and those reported for ox-choroid melanin, pigments isolated from black and red human hair, and for synthetic melanins prepared from tyrosine by the action of potato tyrosinase (*10*) (Fig. 2).

The present data would suggest, therefore, that homogentisic (hydroquinoneacetic) acid can be converted, in the presence of molecular oxygen and at physiological *p*H's, to the corresponding colored quinone, 1,4-benzoquinone-2-acetic acid. This appears, in turn, to be further oxidized to a more complex structure(s) having an absorption spectrum unlike those ascribed to certain of the naturally occurring melanins and to pigmentary material obtained by addition of alkali to homogentisic acid solutions.

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Phototropic Auxin Redistribution in Corn Coleoptiles

Went and Thimann (*1*) have suggested several possibilities to account for the unequal lateral distribution of auxin found in plant parts which have been exposed to unilateral light. Among these are (i) light-induced auxin inactivation, resulting in a lowering of the auxin concentration on the lighted side; (ii) light-induced inhibition of auxin synthesis, having the same effect; and (iii) light-induced lateral transport of auxin from the light to the dark side. Early workers (*2*, *3*) felt that lateral transport of auxin played a major role in phototropic curvature. More recently, their conclusions have been reëxamined, and it has been suggested (*4*) that although the results cited provide support for the concept of lateral transport, it has not been unequivocally demonstrated. Studies by Galston and his associates (*5*, *6*) on light-induced inactivation of indole-3-acetic acid (IAA) have provided considerable evidence that photoinactivation of auxin may play a major role in phototropism.

In the present experiments (*7*), the auxin distribution in coleoptiles of corn (*Zea mays*, var. Burpee Snowcross) was studied in the dark and in unilateral light to determine the relative roles of the three afore-mentioned possibilities in effecting an auxin differential.

Corn seeds were surface-sterilized for 3 minutes in 3 percent Purex, rinsed in deionized tap water, and soaked for 4

hours. They were then germinated on moist filter paper under conditions of constant temperature (25° ± 0.5°C) and humidity (85 ± 1 percent). Only dim red light was used. When the primary roots were of sufficient length, the seeds were set in plastic trays, with the roots extending through holes into deionized tap water.

Preliminary experiments indicated that the coleoptiles showed a maximum growth rate when they were 90 hours old. Further experiments, using standard auxin diffusion techniques (*1*) with 4-mm coleoptile tips and standard agar blocks (8 by 11 by 1 mm), indicated that auxin production was also roughly maximum at 90 hours. Therefore, plants of this age, selected for coleoptiles approximately 16 mm long, were used in all subsequent experiments.

The phototropic sensitivity of coleoptiles of plants of this age was then determined. In three separate experiments, intact plants were placed in unilateral light. The measured light intensity at the coleoptiles was 21.5 meter-candles. Out of a total of 20 plants, all but six showed perceptible positive curvature at the end of 1¼ hours of light, and all showed curvature after 1½ hours. By the end of 3 hours, curvatures varied between 29° and 84°.

Further auxin diffusion experiments were done using 4-mm excised tips to determine the effect of unilateral light on the amount of auxin recoverable. The amount of auxin obtained from tips kept in the dark was compared with the amounts obtained from tips kept in the light for 1 to 3½ hours. Bioassay of the agar blocks from these diffusions, by the standard *Avena* test (*1*) showed no significant difference in the amount of diffusible auxin obtained from the illuminated tips, as compared with the dark controls (see Figs. 1*a* and 1*c*). Thus, if light-induced auxin destruction or inhibition of synthesis were reducing the amount of auxin diffusing from the light side of the coleoptiles, auxin production must be simultaneously increasing on the dark side to account for these results.

In order to determine the importance of light-induced lateral transport in determining the auxin differential associated with phototropic curvature, the technique of Went (*3*), and a modification, were applied to the corn coleoptile tips. The various diffusion experiments done are illustrated in Fig. 1. First, auxin was allowed to diffuse from sets of three intact tips into standard agar blocks for 3 hours. One such set was kept in the dark (Fig. 1*a*), and a second was kept in unilateral light, as described (Fig. 1*c*). Second, auxin was allowed to diffuse from sets of three tips which had been bisected longitudinally, under similar conditions (Figs. 1*b* and 1*d*). Third, six

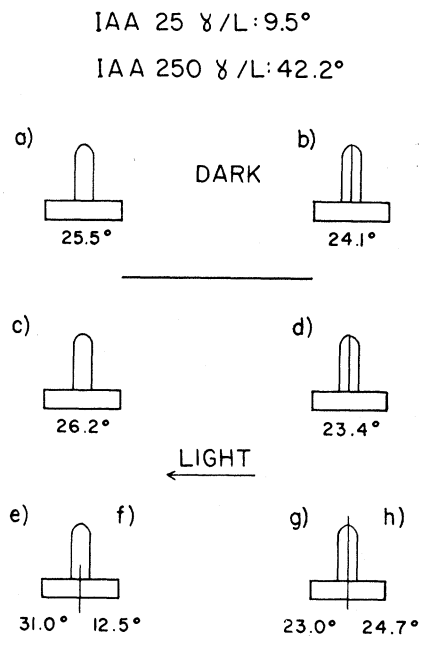

Fig. 1. Auxin diffusion experiments done with corn coleoptile tips. All diffusion times were 3 hours. (*a*) Three intact tips, dark; (*b*) three totally split tips, dark; (*c*) three intact tips, light; (*d*) three totally split tips, light; (*e*) six partially split tips, dark side; (*f*) six partially split tips, light side; (*g*) six totally split tips, dark side; (*h*) six totally split tips, light side. The numbers indicate amount of auxin obtained by diffusion into agar blocks, expressed as degrees of *Avena* curvature.

tips were partially split from the base to a depth of 1 mm. The bases were then separated by a No. 0 cover slip supported vertically between two microscope slides, in such a way that each half of each tip was in contact with a single standard agar block (Figs. 1*e* and 1*f*). Thus, each agar block was in contact with the bases of six half tips, the equivalent of the three tips in the previously mentioned diffusions. Finally, six tips were totally bisected, and the halves were placed on either side of a No. 0 cover slip, so that one-half of any given tip would be directly in the shadow of the other half when the experiment was placed in unilateral light. These sets of half tips were likewise allowed to diffuse on standard agar blocks, one on each side of the cover slip (Figs. 1*g*, 1*h*). The rows of partially split and totally split tips were then placed at right angles to the light source, so that the agar block on one side would collect the auxin diffusing from the dark halves of the coleoptiles, and the block on the other side, the auxin from the light halves. After the 3 hour diffusion period (a total light dosage of 125,000 meter-candle-seconds), all tips were removed, and the blocks were assayed by the *Avena* curvature test.

The results of such an experiment, in which the diffusions were done simultaneously, and the agar blocks then assayed simultaneously, are shown in Fig. 1. They are expressed as degrees of *Avena* curvature. The amounts of curvature obtained from standard blocks containing 25 and 250 μg of indole-3-acetic acid per liter are also shown. The standard error for all values shown was ± 1.92° or less.

There are several conclusions to be derived from these results. A comparison of the amounts of auxin obtained from diffusions 1*a*, 1*b*, 1*c* and 1*d* shows first that total splitting of the coleoptiles causes only a slight and insignificant decrease in auxin production; and second, that the amount of auxin produced was approximately the same, whether the tips were in light or dark for the diffusion time. Next, diffusions 1*e* and 1*f*, from partially split tips, show that light caused a strong difference in the amounts of auxin obtained from the light and dark sides, respectively, less auxin coming from the light side. However, auxin production per tip from diffusions 1*e* and 1*f* is only slightly less than the auxin production per tip from any of the controls (1*a*, 1*b*, 1*c*, and 1*d*). (On three other occasions, production per tip was the same as that of the controls.) These results concur with the observations of Went (*3*) for *Avena* coleoptiles. Perhaps most striking are the results obtained from diffusions 1*g* and 1*h*. When a total barrier is placed between the light and dark halves of the tips, there is no longer any difference in auxin yield between the two halves. As with the partially split tips, the total amount of auxin per tip did not deviate significantly from that of any of the controls.

Although it is possible to show only the results of a single experiment in this figure, the experiment has been repeated on four occasions, with comparable results.

It is felt that these results strongly suggest the following: under the conditions of light intensity, temperature, and humidity used, and with the variety of corn used, the lateral differential of auxin found in coleoptile tips exposed to unilateral light could be brought about only by light-induced lateral transport of auxin from the light to the dark side.

Three lines of evidence support this contention: first, 3 hours of light failed to reduce the amount of auxin produced by intact or totally split tips. Second, when the tips were partially split, a significant difference is noted between the amount of auxin diffusing from the light and dark sides, but the total amount of auxin produced per tip is either the same or only slightly lower than the amount per tip produced by intact controls. In view of the large amount of auxin obtained from the dark sides of the tips, and the almost total recovery of auxin

shown, it seems unlikely that either auxin destruction or inhibition of synthesis could be responsible for the observed differential. Third, when the tips were totally split, and an impermeable barrier was placed between the halves, unilateral light failed to produce any differential at all. Again, if either auxin destruction or inhibition of synthesis were responsible for the observed differential in the partially split tips, one would not expect a total barrier to make any difference in the amounts of auxin obtained. Thus, diffusions 1*g* and 1*h* should be comparable to 1*e* and 1*f*. The most reasonable explanation for these results is that unilateral light actually induces a lateral movement of auxin from the light to the dark side of the coleoptile, and in this way effects the observed auxin differential.

Further experiments are in progress to determine the effect of a range of light dosages on lateral auxin movement, and to attempt to determine in what part of the tip the lateral transport is occurring.

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24 May 1957

Method of Anesthetizing Large Sharks and Rays Safely and Rapidly

In the course of an experimental study dealing with the influence of the anterior pituitary on mating behavior and reproduction in elasmobranchs, it recently became necessary to handle sharks and rays of considerable size. Of the many tranquilizers and anesthetics that were tried, the narcotic known commercially as M.S. 222, a meta-amino-benzoic acid-ethylester in the form of a methan-sulfonate, proved to be the most useful (*1*).

For a quarter of a century, M.S. 222 has been used by the experimental embryologist as an anesthetic for amphibian embryos and small teleosts. Rothlin (*2*) notes that it is isomeric with anesthesin, 3 times less toxic than Novocain, 10 times less toxic than cocaine, and highly

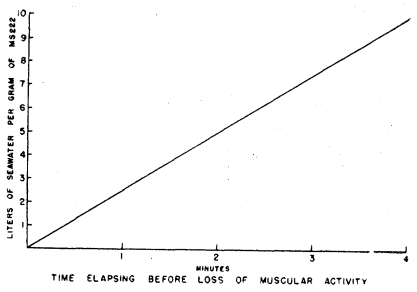

Fig. 1. Relationship between the concentration of M.S. 222 utilized and the rapidity of its action on a young female lemon shark. An initial dose of 100 ml of M.S. 222 solution was sprayed over the gills at the beginning of each experiment. When weaker concentrations were used, additional doses of 100 ml were administered every 60 seconds.

soluble in water. McGovern and Rugh (*3*), using a dilution of 1/3000 on frog eggs and sperms, found that M.S. 222 does not affect sperm motility and fertilizing power, nor does it have any effect on ciliary action; however, it does act quickly on skeletal muscle and effectively immobilizes frog embryos immersed in the solution in 30 to 80 seconds. Schotté and Butler (*4*) point out that M.S. 222 is of great value as an anesthetic in experiments with urodele larvae, since a stock solution of 1/1000 may be sterilized in the autoclave without loss of its narcotic properties and with no increase in toxicity. These investigators have "often kept animals for two consecutive days, and longer, in a 1/10,000 solution without ill effects. Moreover, no cumulative effects have been observed, inasmuch as the same larvae have frequently been submitted to a total duration of 10 days of narcosis within a single month." Many others, including Christensen (*5*), Copenhaver (*6*), Glucksohn (*7*), Rotmann (*8*), Sato (*9*), and Witschi (*10*), have successfully employed M.S. 222 as an anesthetic by simply immersing amphibian embryos or small teleosts in a solution of 1 g of M.S. 222 dissolved in 3000 ml of spring water.

Because of the large size of the elasmobranchs that were investigated, complete immersion in a solution of M.S. 222 was out of the question. An alternative method of utilizing this narcotic was therefore developed, and because of the ease with which it is applied while the fish is still vigorously straining at the hook or harpoon in the water alongside the boat, because its action is dramatically prompt, and because recovery after narcotization is invariably complete, we believe our method may be of some interest and value to others (*11*).

For the most rapid results, a concentration of 1/1000 (1 g of M.S. 222 in 1 lit of sea water) is utilized. On smaller species, 100 ml, and on larger fish, up to 1 lit, of this solution is introduced into the mouth of a shark or the spiracles of a ray and sprayed over the gill exits of the pharynx by means of a water pistol, rubber-bulb syringe, or small, pump-type hand sprayer. During the period of application, the head of a large fish should be held above the level of the water by means of a gaff or the leader that is attached to the hook or harpoon point; smaller fish may be temporarily removed from the water. The drug is quickly absorbed by the gills, and its action is rapid. Within 15 seconds the M.S. 222 solution begins to take effect and, as a rule, even a 400-pound shark is anesthetized in 1 minute or less. The shark or ray may then be readily handled, either out of or in the water, until the first stage of recovery takes place, within 5 to 30 minutes after the animal is returned to the water; this varies with the size of the elasmobranch and the dosage. Recovery may be delayed with an additional application of M.S. 222 solution at this time or may be hastened by washing the gills with fresh sea water (either by "walking" a large shark, with its mouth kept open, around the pool or by directing a stream of fresh sea water into its mouth).

Figure 1 is based on a series of tests in which concentrations ranging from 1/1000 to 1/10,000 were used on a lemon shark (*Negaprion brevirostris*) that weighed 9½ pounds. A 1/1000 concentration of M.S. 222 solution acted in approximately 20 seconds, while weaker concentrations of M.S. 222 solution acted much less rapidly. When the recommended 1/1000 concentration of M.S. 222 solution is utilized, the size of the dose needed to anesthetize a shark or ray within 1 minute is suggested in Fig. 2.

M.S. 222 has been employed effectively on four genera of sharks and two genera of rays, and, in every case, recovery has been complete and the fish appears to have been quite unharmed by

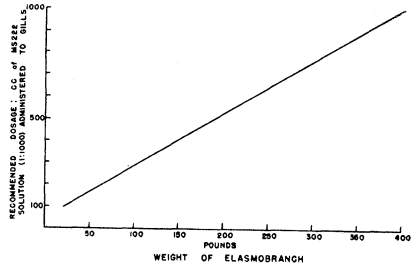

Fig. 2. Size of dose of M.S. 222 solution (1/1000 concentration) recommended to anesthetize a shark or ray in 60 seconds or less. The solution should be sprayed over the gill exits of the pharynx while the head of the elasmobranch is held above the level of the water. If the head remains under water, proportionally stronger concentrations must be utilized.