

on the southwest side of the inner wall of the 1951 cone. Explosions continued at intervals of a few minutes to a few hours. Volcanic bombs were hurled as high as 50 to 60 m. On 12 October two small vents approximately 4 m apart were active in a crater about 6 m in diameter; one vent emitted incandescent lava clots and occasional small lava flows and the other, lava clots and black ash (3). Steam and gases only were emitted from the new vents from 13 October to 11 November.

Small eruptions recommenced at a point about 40 m to the south on 11 November. Another vent formed on 12 November, and intermittent eruptions continued until the morning of 14 November. Ejecta were thrown as high as 60 m, a cone 3 m high was built, and lava flows 50 m long formed. Steam and gases only were emitted from the various vents from 14 November to 1 December.

On 1 December escaping gas formed a fire pillar 3 m high, and the blast ascended 50 m, carrying small amounts of ejecta with it. From 1 December to midnight 18 December, incandescent ejecta, small lava flows, and gas blasts were emitted from the October, November, and other temporary vents.

Explosions began again at 3:27 A.M. on 29 December 1953 and continued intermittently into February 1954. Both the October and November vents were active, and about 12 January an additional vent opened a few meters north of the November vents, and a cinder cone about 40 m high was built by 19 January. The most violent of the 1953-54 eruptions occurred on 27 January 1954, ending at 4:30 P.M. Volcanic bombs reportedly were hurled 800 m high and fell as far as 500 m from the vent. For the first time during the 1953-54 eruption, lava overflowed the 1951 cinder cone crater and partly filled the adjacent larger depression, the depth of which was reduced to about 15 m. The northeast remnant of the 1951 cinder cone was covered thickly by ejecta. Small explosions occurred on 31 January. On 1 February, ejecta were thrown 150 m from the vent, and more lava flowed. Minor activity continued intermittently until about 11 February, and gas outbursts and volcanic tremors continued a few days more. By the end of February, only a little gas issued.

Mihara Yama remained quiescent throughout the remainder of 1954, although steam and other gases in varying amounts were emitted continuously from various parts of the crater. At times steam and gas emission was marked, and billowing clouds rose 100 to 300 m high. Volcanic tremors were recorded at intervals in March, April, June, September, and November. In the first week in November, steam and gas emission increased greatly, and ground temperatures rose in the crater area. Increased amounts of sublimates were deposited in many places in the crater, mostly on or within the 1951 cinder cone. New fissures opened in the remnants of this cone, and some old fissures reopened or widened and began issuing steam. The small cinder cone that was formed in January 1954 was broken by fissures and partly col-

lapsed. On 9 November volcanic tremors ceased and steam and gas emission decreased.

The activity of Mihara Yama in 1953 and 1954 was on a small scale compared with the eruptions of 1950 and 1951. Only small quantities of lava were emitted. The 1953-54 lava was an augite-hypersthene basalt essentially of the same composition as that of the 1950 and 1951 eruptions (4).

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References and Notes

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18 April 1955.

Effect of Reserpine on the Melanophores of Fish

As part of a study of the effects of reserpine (1), the drug was tested for its action on Siamese fighting fish (*Betta splendens*). Abramson and Evans (2) have reported that this fish shows a specific response to LSD-25, and it was our interest to ascertain whether reserpine might inhibit the LSD-25 effect. It can be said at once that, not only did it fail in this, but it acted, in higher dosage levels, as a sedative to *Betta splendens*.

The fish were immersed in 100 ml of aqueous solution of the drug, as Abramson and Evans had done. When the concentration was about 12 µg percent the sedative effect became evident. In this first testing the rather colorless juvenile *Betta splendens* developed within a few hours of immersion a spectacular color display. We have since found this phenomenon to be fairly general, and the effect has been especially studied on several species of fish: *Brachydanio rerio* (zebra fish), *Aequidens portalegrensis* (one of the "acaras"), several *Trichogaster* (gouramies), *Micropodus viridi auratus* (paradise fish), and *Corydoras leopardus* (leopard catfish).

When young fish (2 to 4 cm long) were immersed in the test solution for 6 hr, *Ae. portalegrensis* and *C. leopardus* were clearly distinguishable from untreated controls at a reserpine concentration of 0.4 µg percent. *Ae. portalegrensis* tended to darken when it was left undisturbed but it usually blanched within 30 sec of being excited. Excitement of reserpine-treated fish no longer led to blanching. The effect was still evident but not complete, and somewhat variable, as low as 0.1 µg percent. The effect wore off slowly; traces of coloration more intense than that of the controls were visible in the pectoral fins, sometimes for weeks. The

effect was overcome by cocaine 0.5 mg percent and by ephedrine 45 mg percent and was partially inhibited by ergotamine 0.5 mg percent. It was not inhibited, however, by neosynephrine or pitocin. Previous treatment with desiccated thyroid 1:100,000 for 1 wk did not inhibit the development of color. Injection of epinephrine 0.05 ml 1:1000 led to blanching within a few minutes.

The effect was caused by the dilation of surface and deep melanophores and lipophores, for not only did the black, brown, green, and blue shades become enhanced, but there might, as in *Betta splendens*, be the appearance of reds and golds that were not previously evident in this fish. On the other hand, fish that already had highly dilated lipophores, such as *Carassius auratus* (goldfish) and the red *Xiphophorus helleri* ("helleri"), did not exhibit enhancement of their coloration. The mechanism of dilatation and aggregation of chromophores in fish is still imperfectly understood (3). The speed with which melanophores responded to excitation in *Ae. portalegrensis* suggested that excitement led to epinephrine secretion in the vicinity of the melanophores. Since reserpine inhibited this effect and since only a large dose of ephedrine or injection of epinephrine caused blanching, the effect of reserpine was probably directed to the chromophores themselves rather than through the mediation of the nervous system or by way of hormones such as intermedin or thyroxine.

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References and Notes

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2. H. A. Abramson and L. T. Evans, *Science* **120**, 990 (1954).
3. E. G. Healey, *J. Exptl. Biol.* **31**, 473 (1954); D. L. Fox, *Animal Biochromes* (Cambridge Univ. Press, 1953); G. H. Parker, *Animal Colour Changes and Neurohumours* (Cambridge Univ. Press, 1948).

4 April 1955.

Intermediates in the Biosynthesis of Porphyrins from Porphobilinogen

It has been demonstrated that porphobilinogen (PBG), an α -methylamino monopyrrole, can be used as a substrate for the enzymatic synthesis of a number of porphyrins, including protoporphyrin IX, by cell-free preparations of *Chlorella* cells (1) and of chicken red cells (2). Investigations of early steps in the utilization of PBG in the biosynthesis of porphyrins indicate that a colorless precursor of uroporphyrin, rather than uroporphyrin itself, is the direct intermediate in the biosynthetic chain of the porphyrins (3).

Aqueous extracts of spinach-leaf acetone powder catalyze the disappearance of PBG and the appearance of uroporphyrin I [characterized by the method of Falk and Benson (4)]. These reactions occur aero-

bically or anaerobically. However, the porphyrin does not appear when the reaction is carried on anaerobically in the presence of 0.1M cysteine.

The enzyme porphobilinogen deaminase has been purified approximately 45-fold and has been separated from the other enzymes in extracts of spinach-leaf acetone powder that act in the synthesis of porphyrins from PBG. During the course of the reaction catalyzed by this enzyme, there is a mole-for-mole correspondence between the liberation of ammonia and the disappearance of PBG (as measured by the formation of the *p*-dimethylaminobenzaldehyde complex). This reaction proceeds aerobically, anaerobically, or anaerobically in the presence of 0.1M cysteine. The product of this reaction, in addition to ammonia, is colorless and does not react with *p*-dimethylaminobenzaldehyde. Under aerobic conditions, a small amount of porphyrin may form during the incubation period; for example, in one experiment of 4-hr duration, in which the initial concentration of PBG was 0.96 μ M/ml, 0.94 μ M of ammonia per milliliter was liberated and 0.03 μ M of porphyrin per milliliter accumulated. When this product is allowed to stand at room temperature in air, additional uroporphyrin I accumulates. The optimum pH for the action of PBG deaminase is about 8.2. The enzyme does not catalyze the deamination of β -phenylethylamine, glutamine, or cysteine.

When small amounts of 30 to 40 percent or 40 to 50 percent of ammonium sulfate fractions of spinach-leaf acetone powder extracts are added to a solution containing the colorless product described in the preceding paragraph (but not containing detectable PBG), uroporphyrin I is formed rapidly via an intermediate that is characterized by a strong absorption maximum at about 500 m μ . On the other hand, when a solution containing the colorless product is incubated with a preparation of frozen and thawed *Chlorella* cells (1), usually relatively little uroporphyrin is recovered; but porphyrins with from three to seven carboxyl groups per molecule have been identified on paper chromatograms of the porphyrin products. Coproporphyrin has usually appeared as the major product. From these experiments, it appears that the colorless product, or something derived from it, may serve as a substrate for enzymes that mediate the decarboxylation of porphyrin precursors.

Chlorella preparations appear to be incapable of utilizing uroporphyrin I or III as substrates for the synthesis of porphyrins with fewer than eight carboxyl groups per molecule. This observation indicates that the colorless enzymatic product described here is not converted, for example, to coproporphyrin via uroporphyrin.

Uroporphyrins and very small amounts of coproporphyrins have been recovered after incubation of uroporphyrinogen I or III (prepared by the reduction of the corresponding porphyrins with palladium and hydrogen in alkaline solution) with *Chlorella* preparations. The relatively low yields of copropor-