The action was limited to the brain by the "close arterial" injection into the common carotid artery of the lightly anesthetized cat. Obviously, under these circumstances the serotonin penetrated the blood-brain barrier in order to inhibit the ipsilateral cortical synapses, as was indicated by the reduction in the cortically recorded action potential signaling the evoked response.

We were impressed by the high potency of this action. Serotonin proved effective in doses of as little as 1 μg/kg, and it was 20 times more potent than the next most active natural synaptic inhibitor that we had previously described—namely, adrenaline. The high potency appears to be characteristically cerebral, since the synaptic inhibition we had already recorded in the ciliary ganglion (*2*) required about 75 times the dose. Having dealt only with exogenous serotonin, one could properly ask for a demonstration of the action of endogenous or *in situ* serotonin. We believe that we have given this with the following data.

The action of serotonin is short-lived, because of its great susceptibility to monoamine oxidase (*3*). We reasoned that, if serotonin is naturally present at synapses, we should be able to make its action apparent by inhibiting cerebral monoamine oxidase (MAO), and thereby accumulating *in situ* serotonin until it reaches the threshold for synaptic inhibitory action. This we have done with iproniazid. On intracarotid injection, it reproduces the picture of serotonin action—namely, a reduction of both the evoked responses and the electrocorticogram recorded from the same electrode. As in previous work, we have evoked potentials in the optic cortex of the cat by initiating transcallosal impulses (*1, 4*). That the inhibition of evoked responses and of the electrocorticogram is the result of a poisoning of monoamine oxidase and consequent *in situ* accumulation of serotonin is borne out by the determination of cerebral monoamine oxidase at the time of maximum synaptic inhibition indicated electrically.

The data are displayed in Fig. 1. The monoamine oxidase titer of the injected hemisphere is expressed as percentage of the titer in the opposite or control hemisphere. The evoked potential and electrocorticogram findings are indicated by appropriate symbols at the base of each bar. The left-hand side of the diagram shows the influence of saline control injection. It is evident that there is no change in the electrocorticogram, which was the only electric record taken in this set of controls, and that the variation in monoamine oxidase content of the two hemispheres is relatively small. The extremes of this variation are extended by dotted lines to overlap the right-hand half of the figure, which indicates the data obtained with iproniazid. It is apparent that at the height of synaptic inhibition there is, on the whole, a reduction of monoamine oxidase on the injected side considerably exceeding the natural variations. Similar data were obtained by comparing subelectrode biopsies from the recording hemispheres with the symmetrical points on the opposite sides, but the results were not quite so consistent, probably because of the difficulty in obtaining subelectrode plugs containing identical proportions of the higher monoamine oxidase containing gray matter and the white matter on the two sides.

The direction of these preliminary findings is clear. However, substantiation by further numbers is required to determine whether the instances where electrically indicated inhibition is accompanied by a change in monoamine oxidase titer on the injected side that fails to exceed normal variations are, in fact, due to an initial asymmetrical monoamine oxidase distribution favoring the control side and therefore minimizing the apparent difference induced by iproniazid between the injected and control hemispheres.

This, and the influence of possible leakage across the brain of iproniazid in a few experiments, as well as the safety factor for monoamine oxidase, must be determined. We are now engaged in the final step of simultaneously measuring brain serotonin and monoamine oxidase activity at the time of synaptic inhibition brought about by intracarotid injection of iproniazid.

Since we have shown that many chemical psychotogens are synaptic inhibitors, serotonin or some similar substance seems a likely candidate for the role of an endogenous psychotogen acting by distorting synaptic equilibrium (*5*). That such a distortion could also sometimes be therapeutic is suggested by the recent report of improvement obtained by iproniazid administration in mental disturbance characterized by marked depression (*6*).

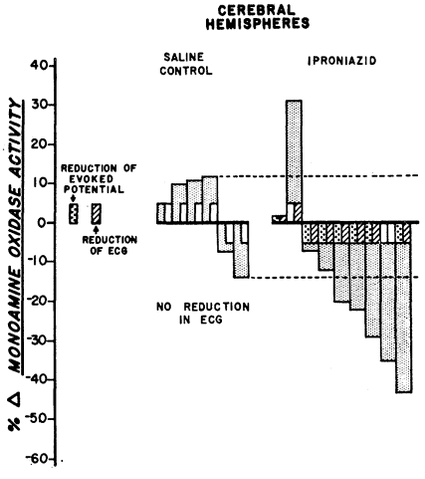

Fig. 1. Correspondence of differential iproniazid inhibition of monoamine oxidase and electric activity in brain. Iproniazid (10 mg/kg) injected into the common carotid artery of a cat anesthetized with sodium pentobarbital.

MELVYN I. GLUCKMAN
E. ROSS HART
AMEDEO S. MARRAZZI
Veterans Administration Research Laboratories in Neuropsychiatry, Pittsburgh, Pennsylvania

References

1. A. S. Marrazzi and E. R. Hart, *Science* **121**, 365 (1955).
2. ———, in I. H. Page and J. W. McCubbin, *Circulation Res.* **1**, 358 (1953).
3. E. A. Zeller and J. Barsky, *Proc. Soc. Exptl. Biol. Med.* **81**, 459 (1952).
4. A. S. Marrazzi, *Science* **118**, 367 (1953).
5. A. S. Marrazzi and E. R. Hart, *J. Nervous Mental Disease* **122**, 453 (1955).
6. J. C. Saunders, N. Radinger, N. S. Kline, *Soc. Biol. Psychiat.*, in press.

2 July 1957

On the Nature of the Pigment in Retinal Pigment Epithelium

Although there have been no biochemical studies of the nature of pigment granules in the retinal pigment epithelium, it has been assumed that the pigment is melanin. This assumption is presumably based on the failure of pigment synthesis in the retinal pigment epithelium of the complete albino. Also, Miescher, in 1923, demonstrated "dihydroxyphenylalanine oxidase" by histochemical methods in the retinal pigment epithelium of the chick, rabbit, and guinea pig (*1*). In some recent manometric studies of homogenates and pigment granules from the retinal pigment epithelium of embryonic chicks, we have demonstrated the presence of the melanin-synthesizing enzyme, tyrosinase (*2*).

The retinal pigment epithelium, derived from neural ectoderm, is a cuboidal epithelium which lies immediately to the outer side of the layer of rods and cones. Pigment granules in human retinal pigment epithelium first appear in the fourth week (5 to 6-mm stage), and by the fifth week (10-mm stage) full pigmentation of the retinal pigment epithelium has occurred. Except for an increase in area, the retinal pigment epithelium remains essentially the same throughout life.

Although retinal pigment epithelium is intimately related to the rods and cones, it is easily separated as a delicate black membrane. Thus it is possible to utilize relatively pure retinal pigment epithelium in enzymic studies with substrates (tyrosine, cresol) that are known

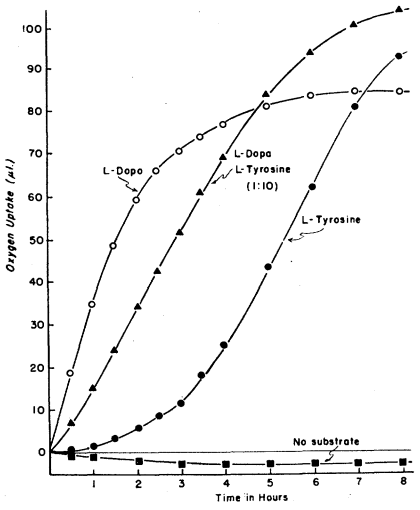

Fig. 1. Enzymic oxidation of L-tyrosine and L-3, 4, dihydroxyphenylalanine (L-DOPA) by homogenates of the retinal pigment epithelium of Rhode Island Red chick embryos. Total amount of substrate, 1.77 µmole.

to form melanin in plants, insects, and chordates (*3*).

For our studies, we used retinal pigment epithelium dissected from eyes of the Rhode Island Red and Black Australorp chick embryos. Homogenates of pigment epithelium from approximately 20 eyes of 9- to 12-day embryos were used in each Warburg flask. In the presence of the homogenates from both species, L-3,4-dihydroxyphenylalanine (L-DOPA) was immediately oxidized without any detectable induction period (Fig. 1. The homogenates catalyzed the oxidation of L-tyrosine only after an induction period which was shortened by the addition of a small amount of L-DOPA (Fig. 1). Four atoms of oxygen and 5 atoms of oxygen were consumed per molecule of L-DOPA and L-tyrosine, respectively. The pigment epithelium homogenates did not catalyze the oxidation of *p*-cresol, catechol, or D-tyrosine. Tyrosinase activity was completely inhibited by the addition of 0.001*M* 4-chlororesorcinol or sodium diethyldithiocarbamate.

By a technique similar to that of Kertesz (*4*), copper was removed by dialysis in the presence of potassium cyanide. The metal-free enzyme was without activity on L-tyrosine or L-DOPA, but addition of trace amounts of copper restored the catalytic activity against L-DOPA but not against L-tyrosine. The failure of the reconstituted enzyme to catalyze the oxidation of L-tyrosine is similar to the behavior of mammalian tyrosinase following removal and addition of copper (*4*). The trace element, copper, which is necessary for formation of melanin, has been found in the retina of mammals, amphibians, and fish (*5*).

A study of pigment granules isolated from the retinal pigment epithelium of Rhode Island Red embryos at different embryonal stages revealed a change in the level of tyrosinase activity during differentiation. Tyrosinase activity was first detectable on the sixth day and gradually increased, reaching a maximum on the tenth day. Enzyme activity fell abruptly after the 12th day, and no tyrosinase activity was present on and after the 14th day of development (Fig. 2).

With a histochemical autoradiographic technique in which DL-tyrosine-2-C^{14} was used as a substrate (*6*), tyrosinase activity was shown to be present in the retinal pigment epithelium of both a C-57 15-day mouse embryo and a 6-month human fetus (*7*). However, no tyrosinase activity was detectable in the retinal pigment epithelium of adult Rhode Island Red and Black Australorp chickens, the C-57 mouse, and the adult human being.

Avian tyrosinase present in the retinal pigment epithelium appears to be similar to plant, insect, and mammalian tyrosinase in its requirement for copper and in exhibiting an induction period in the oxidation of monophenols which can be abolished by addition of small amounts of diphenols (*3*). The specificity of avian tyrosinase for tyrosine and DOPA places it in the class of tyrosinases found in mammalian malignant melanomas (*8*). Plant tyrosinases, being less specific than mammalian tyrosinase, readily catalyze the oxidation of cresol and catechol as well as of tyrosine and DOPA.

It appears from these biochemical studies that the pigment in the retinal pigment epithelium is formed during differentiation by the enzymic oxidation of tyrosine to melanin, catalyzed by tyrosinase attached to cytoplasmic pigment granules. Since avian retinal tyrosinase exhibits some similarities to mammalian tyrosinase present in mouse and human malignant melanomas, it may be useful in elucidating some of the biochemical factors that regulate tyrosinase activity in normal mammalian melanoblasts during differentiation.

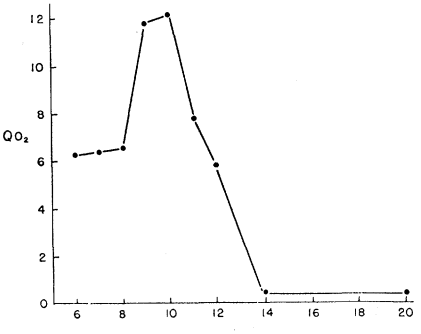

Fig. 2. Changes in tyrosinase activity of melanin granules isolated from the retinal pigmented epithelium of the Rhode Island Red chick during embryonal development.

MASAMITSU MIYAMOTO
THOMAS B. FITZPATRICK
Division of Dermatology, University of Oregon Medical School, Portland

References and Notes

1. G. Miescher, *Arch. mikroskop. Anat. u. Entwicklungsmech.* **97**, 326 (1923).
2. This investigation was supported by the Damon Runyon Fund and U.S. Public Health Service grant C-2408.
3. H. S. Mason, *Advances in Enzymol.* **15**, 105 (1955).
4. D. Kertesz, *J. Natl. Cancer Inst.* **14**, 1081 (1954).
5. J. M. Bowness *et al.*, *Biochem. J.* (*London*) **51**, 521, 530 (1952).
6. T. B. Fitzpatrick and A. Kukita, *J. Invest. Dermatol.* **26**, 173 (1956).
7. A. Kukita and T. B. Fitzpatrick, unpublished data.
8. A. B. Lerner *et al.*, *J. Biol. Chem.* **191**, 799 (1951).

29 May 1957

Kojic Acid—a Convulsant

The mode of formation of kojic acid by microorganisms (*1*), its great chemical reactivity (*2*), and the fact that it is further metabolized by microorganisms suggested to one of us (T.E.F.) that kojic acid might be metabolized by multicellular organisms and, perhaps, also by the diabetic animal (*3*). In the dogs studied by him, the intravenous injection of 150 mg/kg of body weight produced a marked toxic response, including convulsions.

In our studies, mice, guinea pigs, and rabbits, as well as dogs, were employed. Three routes of administration were utilized: subcutaneous, peritoneal, and intravenous. Mice were employed in the intravenous survey experiments (to determine the effective convulsive dose) and in the anticonvulsive studies (*4*). Aqueous solutions adjusted to *p*H 7.0 were employed in all experiments and, in the toxicity experiments, all doses were injected within 20 seconds (*5*).

The administration of large doses of kojic acid (1000 mg/kg) to two dogs caused death to both, yet evidence of kojic acid excretion in saliva and urine, even if indirect, was thereby secured. The nonspecific ferric chloride reaction was used. The CD_{50} and the LD_{50} in mice are approximately 350 and 500 mg/kg, respectively.

The gross external manifestations of kojic acid administered to mice, guinea pigs, rabbits, or dogs are generally alike and are similar to the well-known effects of Metrazol.

Because in dogs the toxic manifestations of kojic acid in dosages of 200 to