type in two other patients with the Ph^1 chromosome. It may be that the Ph^1 deletion varies in size or location from one CML patient to another, or that Mrs. I has an inversion on this chromosome so that the 6PGD locus is deleted. Alternatively, the deletion of the 6PGD locus might affect another chromosome, but the deletion is too small to be detected with currently available cytogenetic techniques.

Other less likely genetic mechanisms include gene inactivation or generalized hypermutability. Inactivation, such as that occurring at X-linked loci, does not occur normally at the 6PGD locus (*10*). Furthermore, the two CML patients with A-B types show that inactivation is not a general phenomenon of this disease, since the intermediate electrophoretic band represents a hybrid of A and B subunits produced within individual cells. Hypermutability was not observed at any other loci, and there were no apparent changes in ABH antigens, such as are observed in some cases of acute leukemia.

If similar results should be obtained in other patients with CML who have the Ph^1 chromosome, the argument for localization of the 6PGD locus on the G-group chromosome would be strengthened. However, only one-half of the A-B heterozygotes with the postulated deletion of the 6PGD locus would be hemizygous for Pd^B. The other half would be hemizygous for Pd^A and would only be detected if there were an anomalous inheritance pattern in their parents or children. Study of 6PGD in patients with trisomy-21 who have A-B phenotypes has potential value in resolving the problem, provided the Ph^1 chromosome is the "21st" chromosome.

The observed aberration appears to involve all (or a great majority) of Mrs. I's red blood cells and granulocytes. Since the most likely explanation for this aberration lies in a genetic mechanism, the observation provides additional support for the earlier conclusion (*3*) that CML has a clonal origin and that granulocytes and red blood cells have a common precursor cell which is the site of the malignant transformation.

P. J. FIALKOW, R. LISKER, J. DETTER, ELOISE R. GIBLETT, C. ZAVALA
Departments of Medicine and *Genetics, University of Washington*, and *King County Central Blood Bank, Seattle*, and *Department of Genetics, Instituto Nacional de la Nutrición, México, D.F.*

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Pars Intermedia: Unitary Electrical Activity Regulated by Light

Abstract. *In the pars intermedia of frogs in the dark two types of spontaneously firing neuronal units have been found; one can be inhibited by and the other is indifferent to increases in illumination. The receptor for the light-inhibited units appears to be the pineal organ. Transection experiments indicate that the axons to the two kinds of units in the pars intermedia are separately grouped in the infundibular floor.*

Pigmentary adaptation in vertebrates requires a system that couples changes in values of environmental light with eventual responses of pigment cells in the skin. The best explored phase of this system involves the release of intermedin or melanocyte stimulating hormone (MSH) from the pars intermedia of the pituitary gland. This hormone directly evokes adaptive changes (darkening) in integumentary pigment cells (*1*), and its action has been well explored. However, the coupling of MSH secretion and release to photoreceptive and other nervous mechanisms is not so well understood. Current research indicates that release of MSH may be under direct inhibitory control of monoaminergic axons that innervate the pars intermedia and synapse with secretory cells (*2*). Stimulatory as well as inhibitory relations of innervation to secretion of MSH have been claimed (*3*), but most experimental evidence in Amphibia, at least, favors a tonic type of inhibitory control.

Since current hypotheses concerning control of MSH release require that neurons link photoreceptive function and the pars intermedia, and since electron microscopy reveals numerous synapses between neurons and secretory cells (*4*), we elected to examine the gland for unitary neuronal electrical activity in frogs.

We recorded such unitary activity from the pars intermedia through microelectrodes (Fig. 1, A and B) and established a lack of this activity in the other large epithelial lobe of the pituitary gland, the pars distalis (Fig. 1C). Furthermore, some of the pars intermedia electrical potentials were shown early in this analysis to be responsive to changes in background illumination (Figs. 1B and 2A).

Seventy-eight adult frogs (*Rana pipiens*) of both sexes, weighing 25 to 60 g, were used. Each frog was lightly anesthetized with ether, immobilized by intramuscular *d*-tubocurarine chloride (1.0 mg per 100 g of body weight), and placed on its back in a transparent plastic frame. Bone and connective tissue were quickly removed from the ventral hypothalamic region under a dissecting microscope, and with minimal bleeding.

A stainless steel microelectrode [electrolytically sharpened, insulated except at the tip (*5*)] led electrical impulses

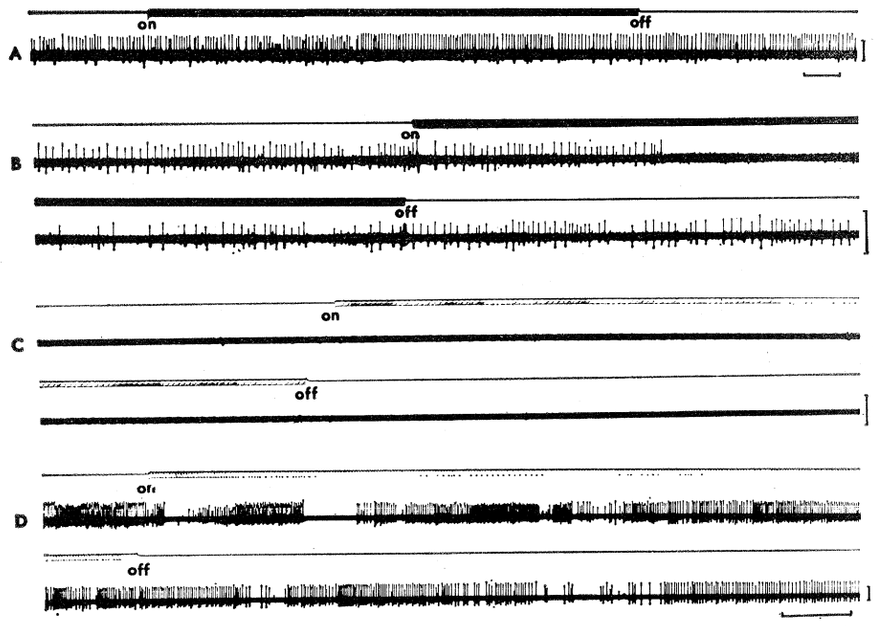

Fig. 1. Spike potential recording from the pars intermedia and the pars distalis and their modification following photostimulation in frogs. In A, B, C, and D, the records are in pairs; one line (marked "on" and "off") indicates the period of light stimulation; the other line is a photograph of the oscillograph records of pars intermedia spike activity during this same period. For B, C, and D, because of their length, the records are reproduced in two successive pairs of lines instead of one. (A) No change in firing rate in the pars intermedia in intact frog after photostimulation. (B) Development of complete inhibitory effect on unitary discharge in pars intermedia in intact frog after illumination. (C) No unitary firing in the pars distalis in an intact frog, and no change evoked by illumination. (D) Transient inhibitory effect of illumination on unitary activity in the pars intermedia in an animal in which the optic tracts were cut and the pineal body was removed. (E) A single-spike recording of unitary discharge from the pars intermedia on an expanded time scale. Solid line over the recording indicates duration of single photostimulation. Calibration: horizontal, 5 seconds in A to D, 25 msec in E; vertical, 200 μv in all.

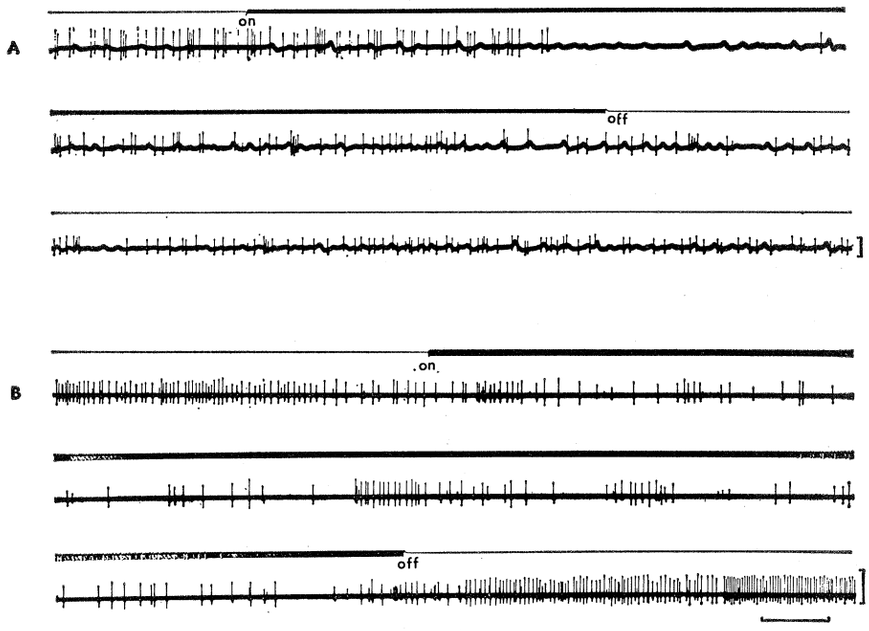

Fig. 2. Effect of optic tract section on photically evoked inhibition in single unitary activity of the pars intermedia. Recordings from the same animal but different units. Photically induced inhibition occurred after optic tract section, but not in the original degree. (A) Before optic tract section; (B) 30 minutes after optic tract section. Calibration: horizontal, 5 seconds; vertical, 200 μv.

from the pars intermedia to a preamplifier (Grass P-5) and Tektronix 502 oscilloscope; impulses were recorded on an oscilloscope camera (Grass C-4). Light stimuli were given by a Grass photostimulator (PS-2) placed 50 cm from the head. Stimuli were constant in intensity (minimal No. 1 setting of instrument that delivers approximately 6×10^6 horizontal candlepower at setting No. 16), and were 50 seconds to 5 minutes long.

Experiments were done in a darkened room regulated at near 21°C; 60-second intervals separated successive photostimulations. The background under the animal holder was alternately changed from black to white. Additional procedures used in particular animals were: section of both optic tracts, eye enucleation, section of the hypothalamo-hypophysial tract just anterior to the median eminence, extension of this transection laterally, and posteriorly, shielding of the brain (except the recording site) from light with a black plastic strip, and removal of the pineal body.

Analysis of 19 single spike potentials from the pars intermedia showed that they are relatively prolonged (Fig. 1E), having a mean duration (initial deflection to terminal deflection) of 17.8 $\pm$ 1.3 msec and a range of 9 to 22 msec. Such slow potentials have been held to be characteristic of neurosecretory neurons (*6*).

Spontaneously firing neurons in the pars intermedia of otherwise untreated frogs are relatively evenly divided between those that are indifferent to external light changes and those that are inhibited. Of 92 such units studied, 42 were inhibited and 50 were indifferent; changing the background from white to black made little apparent difference. A middle-section of the hypothalamo-hypophysial neuronal tract just anterior to the median eminence interrupted all light-inhibitable axons in 15 frogs, but permitted at least some of the light-indifferent fibers to retain electrical activity (12 light-indifferent units were recorded, but no light-inhibitable ones were detectable). Extending cuts laterally and posteriorly interrupted all remaining recordable electrical activity in the pars intermedia in four frogs. The eye is clearly not the photoreceptor for the light-evoked inhibition of pars intermedia electrogenesis, but the pineal organ is obviously an important element in this response. Enucleation of both eyes (seven frogs) or bilateral otpic tract section (seven frogs) still per-

mitted recording of eight light-indifferent spontaneous units and 23 light-inhibitable units. Section of the pineal organ, in addition to optic tract section in the seven frogs mentioned, eliminated light-inhibitable units. Of 15 units recorded in such doubly lesioned animals, 14 were light-indifferent and one was questionably light-inhibitable. It is particularly significant that, in the experiment in which shielding of the ventral surface of the brain from light blocked photic inhibition in the pars intermedia of seven frogs, the eyes were intact.

The extremely long (approximately 30-second) latency of the electrical response in the pars intermedia to external changes in illumination suggests that a humoral step is involved in the response. Since the recording of the final electrical phenomenon is apparently from axonal structures within the pars intermedia, it is most likely that the humoral step is located outside the gland. The nature and locus of such a humoral step is unknown. Its existence can only be presumed from these data, but a search for it is very important.

Electrophysiological techniques, applied here to the study of the pars intermedia, have yielded information that characterizes several previously unsuspected properties of the vertebrate pigmentary response. These data open completely new avenues of inquiry and we hope that they will stimulate and orient further fruitful research into the mechanisms for pigmentary adaptation.

KIYOSHI OSHIMA
AUBREY GORBMAN

Department of Zoology,
College of Arts and Sciences,
University of Washington,
Seattle 98105

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Pituitary-Adrenal Influences on Fear Responding

Abstract. *In a passive avoidance situation, hypophysectomized male rats show less fear than normal rats, whereas adrenalectomized rats show greater fear than normals. These results probably occur because hypophysectomized rats lack adrenocorticotrophic hormone, which increases arousal or emotionality, whereas adrenalectomized animals lack certain adrenal steroids, which inhibit excitatory effects. The results indicate that adrenocorticotrophic hormone and certain adrenal steroids have opposite effects in regulating fear-motivated behavior.*

Administration of adrenocorticotrophic hormone (ACTH) can maintain fear-motivated behavior in normal rats (*1*). Miller and Ogawa (*2*) demonstrated that ACTH prolonged shuttle avoidance responding in adrenalectomized subjects, an indication that this hormone can affect behavior independently of the adrenals. In the experiment reported now, we examined the possibility that ACTH itself has an "excitatory" or "arousing" effect, particularly in augmenting fear responses. We also considered that one action of adrenal steroids released by ACTH might be to shut down or inhibit this excitement, since the pituitary-adrenal system would then influence excitability via a rather elegant feedback circuit—stressors would cause the release of an excitatory hormone (ACTH) that would initiate its own "shut-off" mechanism (steroids). Certain steroids might act by reducing excitability directly, or by inhibiting ACTH since steroids inhibit ACTH secretion when either their blood concentration increases (*3*), or they are introduced directly into the brains of rats (*4*). Moreover, observations that plasma concentration of corticosterone in rat is not maximum until approximately 15 to 60 minutes after stress begins (*5*) and that the inhibitory effect of steroids on ACTH release does not occur until more than 1 hour after stress (*6*), fit well with the view that adrenal steroids could be acting to restore normal excitability after the release of an excitatory hormone.

Male albino rats (20 hypophysectomized by the vendors, 20 bilaterally adrenalectomized in our laboratory, and 20 untreated) (*7*) were used. Hypophysectomized and adrenalectomized animals were used so that there was no opportunity for release of ACTH or corticosterone to influence the secretion of the other hormone. According to the above conception, hypophysectomized animals, which are not able to release ACTH, should therefore become less afraid in a fear situation than normal rats. Adrenalectomized rats, in contrast, can release ACTH but lack steroids and, thus, should become more fearful than normal rats. The latter effect would arise from the lack of certain steroids themselves or from the lack of their normal negative feedback control over ACTH release, or both. Circulating ACTH is high in adrenalectomized female rats 1 week or more after adrenalectomy (*8, 9*); and ACTH release, which occurs within 10 seconds of stress onset, is also greater in adrenalectomized than in normal rats (*9, 10*). Our adrenalectomies were performed 7 to 10 days before experiment.

Fear was measured in a standard passive avoidance situation. The apparatus consisted of a small chamber (17.5 by 9 cm, Plexiglas walls with grid floors, bars 2.5 cm apart) adjoining a large compartment (36 by 36 cm, same construction as small chamber), with a Plexiglas door between them. On each day of the experiment, each animal was weighed and placed into the small chamber so that the animal faced away from the large chamber. When the door was withdrawn, an electronic timer was activated. On day 1 when the rat stepped into the large compartment, the door was closed, and a 1.0-ma shock was delivered through the grid floor for 1.5 seconds, after which the animal was immediately returned to its home cage.

The next day, the animal was returned to the small chamber and the time that the animal delayed (latency) before reentering the large compartment where it had been shocked was measured. No shock was given on this or on subsequent fear tests. It should be noted that, in the passive avoidance test, an animal must step out of the small chamber rather than remain stationary in order to show loss of fear, so that any debilitation among hypophysectomized animals (they are smaller and less robust than normal animals) would work against their being judged less fearful. After the initial test, animals were rested for 2 days and then tested again on each of the 2 succeeding days. If, on any trial, a subject did not step out of the small chamber within 5 minutes, the trial was terminated. Throughout the experiment, ani-