Pigmented Nevi and Malignant Melanomas as Studied with a Specific Fluorescence Method

Abstract. *A specific fluorescence is developed in melanocytes, nevus cells, and cells of malignant melanoma by treatment of the tissue with dry formaldehyde gas. The fluorescence is often stronger in melanocytes adjacent to nevi or melanomas than in normal melanocytes. The strongest fluorescence occurs in cells of malignant melanoma. Among the limited number of compounds that condense with formaldehyde to fluorescent derivatives, DOPA [β-(3,4-dihydroxyphenyl)L-alanine] seems the most likely substance to give rise to the fluorescence observed in these lesions.*

It was reported recently (*1*) that the catecholamines of normal human skin are localized in adrenergic nerves which supply the arrector muscles and the arteries. In that investigation, which was based on a highly specific and sensitive fluorescence microscopic method (*2*) for the localization of certain biogenic monoamines and their immediate precursors, a small amount of specific fluorescence also developed in the epidermal melanocyte system. The compound responsible for this reaction was not identified, but the finding might imply that DOPA [β-(3,4-dihydroxyphenyl)L-alanine] is present in demonstrable amounts in these cells, since no other known precursors of melanin can be detected by the method in question. Melanin formation from labeled tyrosine is evidence of DOPA formation in the cells of certain nevi and melanoma (*3*). In the investigation described here our aim was to examine whether DOPA or a closely related substance is formed in the special cells of nevi and melanomas where it accumulates.

From 24 subjects we obtained 17 pigmented nevi and parts of seven malignant melanomas. Two metastases were included; one of these was from a patient whose primary tumor could not be acquired for examination.

Immediately after removal, the pieces of tissue were quenched in propane cooled by liquid nitrogen and then freeze-dried and treated in dry formaldehyde gas (*2, 4*). This treatment converts the biogenic monoamines as well as their immediate precursors, DOPA and 5-hydroxytryptophan, into fluorescent compounds. The chemical background of the reaction is well understood (*5, 6*). Thus, the catecholamines and other phenylethanolamines with an OH-group in 3- and 4-position condense with formaldehyde to form highly fluorescent isoquinoline derivatives.

Under the conditions of fluorescence microscopy used (*4*), the fluorophores emit a green light as in adrenergic nerves, but when extreme concentrations are present, as in certain chromaffin cells, the fluorescence is yellow. The condensation reaction for 5-hydroxytryptamine and 5-hydroxytryptophan results in the formation of fluorophores that emit a typical yellow light. To differentiate between specific fluorescence—that is, fluorescence developed by the formaldehyde treatment—and unspecific autofluorescence, some pieces of tissue were treated in the same way but without formaldehyde gas. The preparations were embedded in paraffin under reduced pressure, and serially sectioned at 6 to 10 μ. Every fifth section was stained in hematoxylin and eosin to make possible an analysis of fluorescent structures in the light microscope. The rest of the sections were mounted for fluorescence microscopy.

A characteristic feature of many nevi was that an enhanced fluorescence in the green-to-yellow range occurred in apparently morphologically normal melanocytes at the dermo-epidermal junction. The fluorescent material was confined to fine granules in the cytoplasm of the cell bodies and their processes, whereas the nuclei were dark.

In all nevi there occurred rounded or elongated nevus cells with dark nuclei surrounded by a rim of cytoplasm that contained fine granules which displayed a yellow-green fluorescence of varying intensity. A peculiar topographic distribution of the fluorescence was observed: cells with the strongest fluorescence lay adjacent to the surface epithelium, whereas the deeper cell layers showed a gradual and rapid decrease in fluorescence intensity. Thus, the central and lower parts of the cell aggregations lacked specific fluorescence. In stained sections there was no difference between the fluorescent and nonfluorescent cells.

All the nevi examined had two striking features in common: (i) the nevus cells contained varying but often large amounts of a substance which apparently possesses the special and necessary molecular requirements to condense with formaldehyde to form a fluorescent derivative (*5*), and (ii) the fluorescence was increased in many adjacent melanocytes of normal appearance.

The malignant melanomas were characterized by the presence of cells with an extremely intense fluorescence. In all cells the fluorescence was confined to the cytoplasm, the nuclei appearing dark in the fluorescence microscope. In some instances, singular or small groups of fluorescent cells were found at considerable distances from the border of the lesions.

One of the metastases was located subcutaneously near the site of the primary tumor. It was composed of cords of tightly packed cells showing a moderate to intense, yellow granular fluorescence. The cells were rather uniform, rounded or somewhat elongated, and only occasionally had processes. The other metastasis was found in a lymph node. This contained masses of bizarre cell forms, which exhibited a weak to strong granular fluorescence in the green to yellow range.

The identity of the compound that gives rise to the specific fluorescence in nevi and melanomas has not been established. The intense yellow fluorescence often found in nevi and melanomas could be indicative of the presence of a tryptamine derivative such as 5-hydroxytryptophan or 5-hydroxytryptamine. However, in the restricted group of biogenic substances that condense with formaldehyde to form fluorescent products, DOPA seems to be the more probable substance since it is a precursor in melanin synthesis, although it cannot be excluded that an aberration of the pigment metabolism occurring in malignant melanomas might include the formation of catecholamines. The possibility that DOPA is responsible for the fluorescence in the cells of malignant melanoma is supported by the fact that a high rate of melanin formation from tyrosine has been demonstrated in these cells and that DOPA is an intermediate product of the synthesis (*3*). Furthermore it has been observed that large amounts of DOPA are excreted in the urine of patients with malignant melanomas (*7*). In the proliferative phase of junctional

nevi it has been possible to demonstrate melanin formation from labeled tyrosine, but inactive junctional nevi and dermal nevi seem to have a lower amount of tyrosinase (*3*). It seems quite possible that, with the sensitive fluorescence method we used, DOPA could be detected even in cells where the tyrosinase has not been demonstrated.

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Spontaneous Opiate Addiction in Rhesus Monkeys

Abstract. *Spontaneous drug-seeking behavior was established in 3 out of 4 rhesus monkeys that were given unrestricted access to morphine and placebo solutions. The monkeys were kept in their home cages throughout the experiment and were not subjected to conditions of stress; prior addiction was not established by the usual injection procedures. The animals became physically dependent on the drug and showed abstinence symptoms when injected intramuscularly with nalorphine (N-allyl normorphine hydrochloride). This method may be useful for studying individual differences in susceptibility to drug abuse.*

Studies of drug addiction in human populations are complicated by legal sanctions and social restrictions which tend to make addict societies closed and inaccessible to scientific scrutiny. Most investigators of drug addiction in animals have relied predominantly on methods of prior addiction induced by the regular injection of opiates percutaneously (*1*). In those experiments where oral administration has been used and the animals have been allowed free movement in their home cages, the addiction was induced first by providing drugged solutions alone; only after a period of "forced choice" were the animals given a choice between drugged and nondrugged solutions (*2*). Although the spontaneous choice of morphine has been observed in experiments where both water and morphine solutions were provided, the results of such experiments are not generally available in the literature (*3*). Other investigators have used a harness and intravenous canulation for drug administration, the animals first being addicted by injection and then allowed to maintain themselves on the drug by pressing a bar which delivers a dose of morphine intravenously (*4*). Such apparatus obviously abolishes spontaneous choice as an element of drug-seeking behavior.

We have studied the development of spontaneous drug addiction or drug-seeking behavior in four rhesus monkeys (*Macaca mulatta*). The animals were kept in their individual home cages without being subjected to conditions of stress and were given unrestricted access to a placebo (water) and a 0.1-percent solution of morphine sulfate. Food was provided according to a normal schedule. To test for taste preference, two additional monkeys were given access to the placebo and a 0.03-percent solution of quinine. The animals were adolescent males, approximately 18 months in age, weighing from 3.5 to 4.5 kg. The cages were equipped with Plexiglas doors with two nipples fastened into the door and protruding into the cage. The nipples were connected by plastic tubes to two 1000-ml calibrated burettes. Grason-Stadler electronic drinkometers and an Esterline Angus event-recorder were used to record the volume of each fluid taken by the monkeys as well as the number and temporal spacing of their drinking responses. Drug-seeking behavior was defined in terms of the relative number of times the monkeys drank from each nipple, as well as the volumes of each solution ingested.

Initially, a 1-percent solution of morphine was provided by one drinkometer and water by the other. Since very little morphine solution was consumed, we decreased the concentration to 0.1 percent and found that the solution was preferred in increasing amounts in relation to water by three of the animals over an extended period. Within the first week, one animal stopped drinking the morphine solution. The other three animals manifested a variety of abstinence symptoms when injected with nalorphine. Their different patterns of drug-seeking behavior are shown in Fig. 1. The amount of drug taken by animal 1 increased for 120 days; the intake reached a maximum of 520 mg of drug every 24 hours, 10 days after nalorphine precipitated abstinence. Animal 2 increased its intake of morphine and water over an 80-day period; after nalorphine was administered, the intake of both water and morphine declined. At the same time that animal 3 showed a preference for the drug, it reduced its total fluid intake and showed a loss of weight and physical deterioration. When these changes in physical status occurred we discontinued the tests. Nalorphine was given at the end of 80 days to establish that physical dependence had taken place.

The data for the volumes of morphine consumed were evaluated statistically. Analysis of variance for differences among trends for each subject was performed on the data for morphine intake over 80 days (*5*). A mean value for eight intervals of 10 days each was computed, data for the total daily intake of morphine prior to the injection of nalorphine being used. Each 10-day interval was divided into two parts to estimate the variability within each interval. The interaction of subjects and intervals of 10 days was highly significant ($p < 0.0001$; $f = 13.7$; $df = 14/24$). Variation due to differences in linear and cubic trends among subjects accounted for 25.2 percent and 27.4 percent of the total interaction, respectively. Subject differences in quadratic trends explained the largest portion of interaction variance, 46.3 percent. Deviations from these trend differences amounted to only 1 percent of simple effects variation.

The relation between physical and psychological factors in addiction are complex, and differences of opinion exist regarding the relative importance of each factor. It is contended on one hand that addiction or physical dependence begins with the first dose (*6*). Other investigators insist that habituation is distinguished from addiction by the presence of abstinence symptoms, primarily autonomic in origin, which are the hallmark of the addicted state (*7*).

The test of addiction in common use with human subjects is the ab-