7-Hydroxychlorpromazine: Potential Toxic Drug Metabolite in Psychiatric Patients

Abstract. *7-Hydroxychlorpromazine was isolated from liver and urine of psychiatric patients treated with high dosages of chlorpromazine for long periods. These patients developed a purple pigmentation limited to areas of skin exposed to sunlight, as well as opacities of the cornea and lens. Large amounts of melanin were found only in the pigmented skin. It seems likely that a toxic compound is produced by the action of ultraviolet light on a 7-hydroxylated chlorpromazine derivative deposited in skin, cornea, and lens, and that this in turn leads to excessive melanin production and eye changes.*

There are two recent reports (*1*, *2*) of a newly recognized syndrome that occurs in some psychiatric patients who have been given chlorpromazine in high dosages for prolonged periods. Such patients exhibit a disfiguring purple-grey (*1*) or blue-black (*2*) pigmentation of those areas of the skin which are exposed to sunlight. The discoloration frequently first becomes apparent in summer, but does not disappear in winter or after cessation of drug treatment. In addition, many of the affected patients develop corneal and lens opacities (*1*). The "purple people" syndrome was at first thought to occur only in women, but more recently it has also been observed in male psychiatric patients (*3*).

Chlorpromazine is metabolized in man to form a wide variety of derivatives. The *N*-dimethylaminopropyl side chain can be either partially or completely demethylated, or converted to the *N*-oxide, and the phenothiazine nucleus is subject to sulfoxidation and to ring hydroxylation. The hydroxylated or phenolic derivatives can in turn be conjugated with glucuronic or sulfuric acid. Since these alterations can occur singly or in combination, a large number of metabolites are possible theoretically, and some of these may not only account for the pharmacological activity of chlorpromazine but also for its varied toxic effects.

7-Hydroxychlorpromazine was recently identified as one of the metabolites excreted in the urine of persons receiving repeated doses of chlorpromazine (*4–6*). When we observed that authentic 7-hydroxychlorpromazine (*7*) rapidly turned purple both in solution and on paper chromatograms after brief exposure to sunlight, it occurred to us that this metabolite might be responsible for the photosensitive purplish pigmentation of the skin.

Liver was obtained at autopsy from a 52-year-old mentally retarded woman who had received chlorpromazine daily for 6 years before her death in doses as high as 1600 mg. Her face had been purple for more than 2 years, and she had had brownish stellate central cataracts for at least 1 year before her death. A urine specimen and skin biopsies were obtained from a second mentally retarded woman, aged 32, who had been given chlorpromazine for 10 years in doses ranging up to 2800 mg per day. At the time these specimens were obtained, she exhibited a deep purplish-grey color of her face, neck, forearms, hands, and lower legs, while the skin in areas not exposed to sunlight was entirely unpigmented. She also had hazy opacities of her corneas and bilateral central stellate cataracts.

Urine was extracted at *p*H 9.0 with chloroform, and after enzymatic digestion with glucuronidase was extracted first with chloroform and then with *n*-butanol, as described by Posner *et al.* (*4*). Portions of liver and skin were homogenized in water with an omnimixer and were then similarly extracted, both before and after enzymatic hydrolysis with glucuronidase. The concentrated extracts of urine and tissues were chromatographed one-dimensionally on paper in *n*-butanol, ethanol, and water (15 : 2 : 5) (*5*), and spots were visualized by spraying the sheets with 10 percent sulfuric acid in 80 percent ethanol (*4*), with diazotized *p*-nitroaniline, and with ninhydrin-lutidine, or by exposing the chromatograms to ultraviolet light.

7-Hydroxychlorpromazine, as well as unmodified chlorpromazine and chlorpromazine sulfoxide, were identified as free amines in urine. The greater part of the 7-hydroxychlorpromazine in the urine examined, however, was conjugated with glucuronic acid and was extracted into chloroform after enzymatic hydrolysis. Free 7-hydroxychlorpromazine was the only drug metabolite extractable from a 3-g portion of liver obtained from the autopsied patient. No chlorpromazine derivatives could be extracted from either purple or nonpigmented skin obtained from the patient whose urine was studied, but this may have been because less than 0.5 g of skin was available for chemical extraction.

The identification of the 7-hydroxychlorpromazine found in urine and liver was based on the following data. Both the authentic compound and the material isolated from the patients had R_F's of 0.80 to 0.82 in *n*-butanol, ethanol, and water, and both gave the same color reactions with the spray reagents used. These were purple with sulfuric acid (nonphenolic chlorpromazine derivatives yield pink colors), green with diazotized *p*-nitroaniline, and no color with ninhydrin-lutidine. Both the metabolite extracted from urine and liver and authentic 7-hydroxychlorpromazine gave rapidly developing pink-purple spots when paper chromatograms were exposed to ultraviolet light; nonphenolic chlorpromazine derivatives yield weak tan spots much more slowly.

We found that authentic 7-hydroxychlorpromazine was eluted from a 30-cm column of Amberlite CG-50, type 2, at an effluent volume of 168 to 208 ml, when 4*N* acetic acid was pumped through the resin column at a flow rate of 10 ml per hour and at a temperature of 40°C. Under these conditions chlorpromazine sulfoxide was eluted between 105 and 147 ml, while chlorpromazine and nor$_2$chlorpromazine were eluted from the column much more slowly. The material derived from liver and from urine, whose paper chromatographic mobility and color reactions matched those of 7-hydroxychlorpromazine, was eluted from the column between 168 and 208 ml.

The urine specimen studied contained, besides 7-hydroxychlorpromazine, other conjugated phenolic chlorpromazine derivatives which could not be identified positively, since authentic compounds were not available. One of these, present in greater concentration than 7-hydroxychlorpromazine, was ninhydrin positive, indicating that its amino group was completely demethylated. It was eluted from the Amberlite CG-50 column at the same effluent volume as chlorpromazine sulfoxide. Another major conjugated metabolite yielded a blue color when chromatograms were sprayed with sulfuric acid. Spectrophotometric examination at 580 mμ of the urine reacted with sulfuric acid, and comparison with appropriate concentrations of authentic 7-hydroxychlorpromazine, indicated that the patient excreted approximately 1000 mg of phenolic chlorpromazine derivatives per day.

Skin biopsies (2 cm by 3 mm) were

obtained from one patient for histological and histochemical study. A piece of deeply pigmented skin was removed from the neck, and a similar piece of nonpigmented skin was removed from a neighboring area of the chest which had not been exposed to sunlight. These were immediately frozen in isopentane cooled to −160°C with liquid nitrogen. Sections were then cut in a cryostat at −15°C at a thickness of 8 μ.

Hematoxylin and eosin-stained sections of the nonpigmented skin contained no obvious pigment, whereas, in the skin from the pigmented area, there was a marked deposition of a yellow-brown pigment in cells adjacent to capillaries within the superficial layers of the dermis, and to a lesser degree in the basal layers of the epidermis. With Masson-Fontana's silver method for melanin (*8*), the pigment reduced ammoniacal silver to metallic silver so that the heavy deposits in the dermis became blackened and a finely granular deposit throughout the basal layer of the epidermis became visible (Fig. 1*a*). The nonpigmented skin showed only occasional granules in the basal layer by this method.

Tyrosinase (DOPA-oxidase), normally responsible for the conversion of tyrosine to melanin, was demonstrated by the method of Becher *et al.* (*8*), and was present to a marked degree in the basal layer and to a lesser degree in pigment deposits in the dermis of the pigmented skin, yet was almost completely absent in the nonpigmented skin (Fig. 1*b*).

When we used a more specific test for melanin, Lillie's iron-uptake method (*9*), the pattern was similar to that shown by the Masson-Fontana method (Fig. 1*c*). In addition, the pigment proved to be only soluble in 1*N* NaOH, and was bleached by 10 percent hydrogen peroxide in 24 hours.

With Schmorl's reaction (*8*) promelanin was demonstrated in addition to melanin. This reaction gave a great degree of positivity in the basal layer of the pigmented skin, with little reaction in the skin from the nonpigmented area. The pigment was negative according to Perls's Prussian blue reaction for iron (*8*) and when tested by the periodic acid-Schiff reaction. Histochemically this pigment is indistinguishable from melanin and is positive when tested by all those reactions regarded as specific for it.

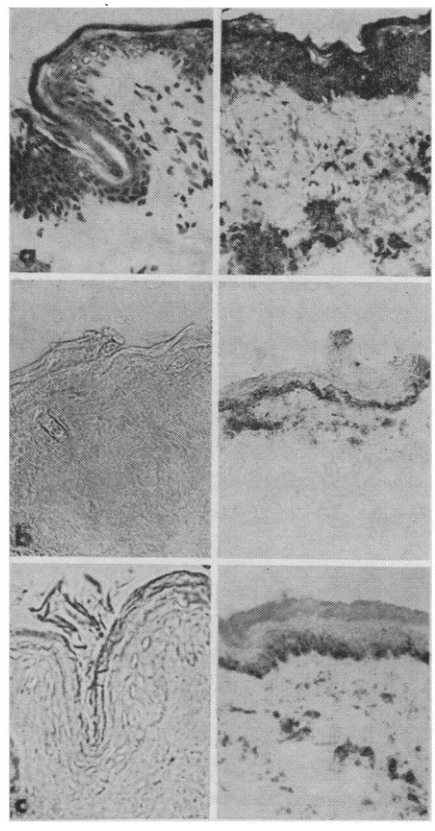

Fig. 1. Photomicrographs (× 80) of sections of nonpigmented skin (left) and pigmented skin (right). *a*, Masson-Fontana method; *b*, tyrosinase reaction; *c*, Lillie's iron-uptake method.

Histologically the deposits of pigment seemed to be located in macrophages adjacent to capillaries and in the basal layer of the pigmented skin. The purple coloration could be seen grossly as though the surface of the skin had been stained, and yet this color was not visible microscopically in unstained sections. It was hard to realize that the uncolored, nonpigmented skin was obtained from the same patient.

We consider it likely that 7-hydroxychlorpromazine, or a further metabolite of this compound, accumulates in the skin, as well as in other tissues of affected persons, and that upon exposure to ultraviolet light the drug metabolite is converted to a purple compound, a transformation which readily occurs in vitro under apparently physiological conditions. The restriction of pigmentation to areas of skin exposed to light suggests that the presence of one or more phenolic chlorpromazine metabolites which have undergone light-induced alteration in their structure is in some way responsible for the observed increase in tyrosinase activity and in melanin production. The fact that the pigmented skin has an unusual purplish hue suggests either that it contains a purple chlorpromazine metabolite in addition to melanin, or that a pseudomelanin having a color distinct from normal melanin has been formed, possibly by polymerization of quinoid derivatives of the phenothiazine nucleus.

The corneal opacities and the central stellate cataracts observed in many of the "purple people" might be explained by the action of ultraviolet light reaching the cornea and lens and effecting a chemical transformation of 7-hydroxychlorpromazine or one of its derivatives. In this connection, a photosensitized keratitis has been reported to occur in calves following the administration of phenothiazine as an anthelminthic (*10*).

Only a minority of mental patients receiving chlorpromazine develop this toxic syndrome, and the incidence of the syndrome varies from one mental institution to another. The extent to which its occurrence is determined by dosage, duration of therapy, amount of exposure to light, sex, and variations in the metabolism of chlorpromazine needs to be determined by further investigation. The possibility that phenothiazine tranquillizers other than chlorpromazine may also give rise to photosensitive skin pigmentation and eye changes should be explored.

Several practical measures to prevent the syndrome suggest themselves. Would chemical substitution at the 7 position in the phenothiazine nucleus yield a compound with desirable ataractic properties, and yet one incapable of metabolic ring hydroxylation? It might be wise for patients requiring large doses of chlorpromazine over long periods to wear dark glasses and not to be permitted outdoors. Special window glass which admits a minimum of ultraviolet light might be installed in the wards of mental hospitals. Most important, the clinical indications for long-term administration of tranquillizers to psychiatric patients should be periodically and very critically reviewed.

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Visual Evoked Potentials as a Function of Flash Luminance and Duration

Abstract. *Computer-averaged evoked potentials were recorded to visual stimuli of constant duration and varying luminance, as well as to flashes whose luminance and duration varied reciprocally. With constant duration, the latency, amplitude, and waveform of the evoked response varied as a function of luminance. The effects of decreasing the luminance on amplitude and waveform of the responses can be balanced by increasing the duration of the flash. This reciprocity between luminance and duration suggests a relationship between apparent brightness and evoked potentials.*

The data from several studies of visual evoked potentials in humans, in which light flashes of constant duration were used, suggest that the waveform of the potentials varies as a function of stimulus luminance (*1*). The use of flashes of constant duration, however, makes it difficult to separate the effect of the physical parameter, luminance, from that of the psychological parameter, apparent brightness, since the latter may vary as a function of both luminance and duration (*2*). It is therefore of interest to relate these physical and psychological dimensions to electrophysiological data.

One method of holding brightness constant while changing physical parameters is suggested by Bloch's law, sometimes referred to as the Bunsen-Roscoe law (*2, 3*). According to this law, the apparent brightness of flashes shorter than some critical period C depends on both luminance and duration. In other words, flashes which vary in these physical parameters can be made to have the same apparent brightness as long as the product of their luminance and duration remains constant, and C is not exceeded. The exact value of the critical period, C, varies with the conditions of observation, but it is usually given at about 100 msec. Using this method, we have investigated the average visual evoked potentials elicited by light flashes varied in both luminance and duration and thereby related to apparent brightness.

Two different stimuli were presented to the fovea of the right eye in Maxwellian view: a semi-circle of 1° 22′ visual angle along its diameter, and a full circle of 2° 6′ visual angle. The light sources were Sylvania R1131C glow modulator tubes, whose luminous intensities were initially equated by means of a photomultiplier display on a cathode ray oscilloscope. The maximum luminance of the circular stimulus and that of the semicircular stimulus was 9000 mlam. Flash duration was controlled by two Grass-S4B stimulators monitored by an electronic counter. The subjects fixated four dim red lines converging upon the stimulus area and pressed a key to trigger each flash arhythmically about once every 2 seconds (*4*). When sufficient stimulations had accumulated, the experimenter terminated that series. Electrical potentials were amplified by a Grass model 6 electroencephalograph and recorded on magnetic tape. The average evoked potentials were obtained with a Mnemotron Computer of Average Transients, a 1-second epoch being used, with a sampling rate of 400 per second. All records presented here are from the scalp over the visual area, 2.5 cm above the inion and 2.5 cm to the right of the midline, with reference to the left ear lobe.

Figure 1 shows the average evoked potentials obtained from one of seven subjects exposed to flashes of constant duration (10 msec) and varying luminance [see Donchin (*5*) for further details]. As flash luminance was reduced over a range of 4 log units, changes occurred in the number, amplitude, and latency of the components of the average evoked potential as well as in the overall waveform. With the stimulus of greatest luminance, namely 9000 mlam, two diphasic waves appear in the average evoked potential (*abc* and *cde* in Fig. 1). Negative peak latencies occur at about 80 and 175 msec (*b, d*); positive peak latencies, at about 120 and 210 msec (*c, e*).

In the average evoked potentials elicited by the circular stimulus, the peak-to-trough amplitude of the first diphasic component (*b–c*) decreases as the luminance is reduced and eventually disappears into the background activity. The peak-to-trough amplitude of the second diphasic component (*d–e*) initially increases as luminance is decreased and reaches a maximum approximately when the first diphasic wave disappears. With further reduction in luminance, the amplitude of this component sharply diminishes, and a long positive wave of 200 to 400 msec develops when the luminance approaches threshold values. The negative peak latencies of both diphasic waves increase with reduced luminance, by approximately 40 msec over 5 log units.

These same trends are apparent when the luminance of the semicircular stimulus is reduced. The waveforms tend to be similar when the

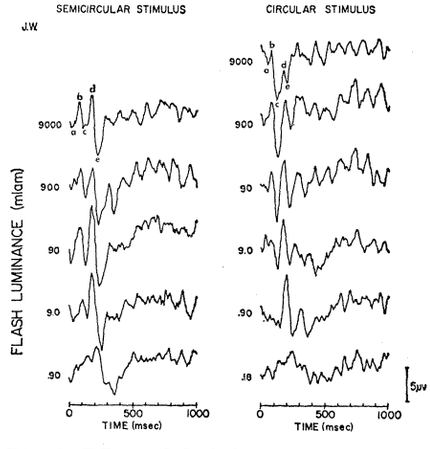

Fig. 1. Effect of flash luminance on average evoked potentials for subject J.W., with semicircular and circular stimulus flashes of constant duration (10 msec). Flash onset occurs at the start of each trace. Each average is based on 100 stimulations. Negativity at visual area in this and subsequent figures is upward. The bottom traces in both columns are responses to stimuli just above subjective threshold; areal differences account for their different luminance values.