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8. Directly elicited action potentials were obtained by inserting two microelectrodes into the same surface fiber 50 to 100 μm apart, one to pass current and the other to record the voltage response. The RMP was set at −90 mv to obtain more homogeneous responses by applying a hyperpolarizing current through the current electrode [T. Narahashi, *J. Cell. Comp. Physiol.* **64**, 73 (1964); E. X. Albuquerque and S. Thesleff, *Acta Physiol. Scand.* **73**, 471 (1968)]. In many cells, the depolarizing current pulse was also delivered with the RMP set at −120 mv to ensure that the pattern of TTX-resistance which we observed was not dependent on the membrane holding potential. The effect of TTX ($1 \times 10^{-6}M$) on directly elicited action potentials was then compared in the two classes of surface muscle fibers to determine whether the action potentials of innervated muscle fibers of partially denervated muscles develop resistance to the blocking action of TTX in a similar way to that observed in denervated muscle fibers.
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Phototoxic Keratoconjunctivitis from Coal-Tar Pitch Volatiles

Abstract. *Roofers working with coal-tar pitch develop burning eyes and conjunctivitis which they subjectively associate with sun exposure. A coal-tar pitch distillate instilled in the conjunctivae of rabbits produced minimal or mild irritation in the absence of ultraviolet radiation, but irradiation with long-ultraviolet produced marked photophobia and severe keratoconjunctivitis.*

Photosensitization reactions induced by exogenous agents have not been clearly shown to cause human eye disease. In this report we describe observations on humans employed in the roofing trade and on New Zealand white rabbits which demonstrate that ultraviolet (UV) radiation of wavelengths present in sunlight, and volatiles from coal-tar pitch, can act in concert to produce injury to the cornea and conjunctiva (keratoconjunctivitis). This injury appears analogous to phototoxic injury to the skin from coal-tar pitch and sunlight (*1*, *2*).

Two groups of U.S. roofers occupationally exposed to coal-tar pitch were examined. Six of 34 roofers in one group and 11 of 17 roofers in the other had clinical signs of keratoconjunctivitis. There were statistically significant ($P < .05$, Fisher's exact test) correlations between the presence of conjunctivitis on examination and occupational exposures to airborne polycyclic particulate organic matter of ≥ 0.20 and ≥ 0.18 mg/m^3, respectively, for the two groups (*3*). In each case the personal exposures represented a time-weighted average value for the entire work period. Affected roofers complained of severe burning in the eyes on exposure to the sun. In addition, the majority of roofers in each group gave a history of severe episodes of probable keratoconjunctivitis in the past, which they related to exposure to pitch vapors or dusts. Other authors have described both acute and chronic ocular changes as a result of human exposure to coal-tar pitch or its components (*1*, *4*, *5*). Susorov (*5*) noted that eye irritation in workers unloading pitch was more pronounced when the work was carried out in clear sunlight.

Table 1. Subjective assessment by 11 white journeyman roofers of the relative influence of environmental variables and different roofing materials on the development of skin and eye symptoms. Symptoms were scored as very bad, 5; bad, 3; occasional, 1; and none, 0.

Variable	Mean score	
	Eye symptoms	Skin symptoms
Sunny day	3.9	2.5
Summer	3.7	2.7
Windy day	2.9	2.7
Very humid	2.8	2.3
Still day	2.0	1.8
Not humid	1.4	1.3
Winter	1.0	1.0
Cloudy day	0.9	1.0
Pitch (about 260°C)	3.6	2.5
Pitch (about 200°C)	2.2	1.6
Asphalt	0.1	0.1
Gravel	0.2	0.1

Fifteen journeyman roofers rated from personal experience the relative influence of certain environmental variables in producing eye and skin symptoms. Selected ratings obtained from 11 white males are shown in Table 1. Although only the skin manifestations are generally considered phototoxic, subjective associations with sunny days or summer were stronger for eye symptoms than for skin symptoms. Four black male roofers gave rankings for eye symptoms similar to those of the white roofers, but only one black reported any skin symptoms. Both eye and skin symptoms were clearly associated with the use of coal-tar pitch rather than other roofing materials and were more pronounced when pitch was used at a higher temperature, where more would be volatilized.

As these observations suggested a role for photosensitization in producing eye irritation, the results of controlled exposures of the eye to UV and pitch alone and in combination were examined in New Zealand white rabbits. The animals weighed between 3 and 4 kg and were maintained in UV-free quarters. Thirty minutes before treatment acepromazine (2 mg/kg) was given subcutaneously and eyelashes were trimmed to a length of 3 mm. One eye of each rabbit was exposed to radiation from a bank of BLB40 fluorescent black lights (predominantly 330 to 380 nm), passed through a window-glass filter to remove radiation below 320 nm. Total radiant exposure was controlled by using a photodosimeter. During irradiation the other eye was covered with an opaque shield and reflective surfaces in the room were covered with black cloth. Roofing coal-tar pitch volatiles were prepared by collecting vapors from a large sample maintained at 200°C, using Romovacek's method (*6*). Gross and biomicroscopic observations of the eyes were made 5, 24, 48 and 72 hours after treatment (longer if abnormalities persisted) by an observer who did not know how each eye had been treated.

Preliminary studies showed that rabbits irradiated shortly after conjunctival instillation of 10 μl of coal-tar pitch distillate to both eyes would tightly close only the irradiated eye within about 3 minutes (4×10^2 joule/m^2 UV) and keep that eye closed until irradiation was ceased. As a result it was necessary to use an eye speculum to keep the lids open during irradiation.

Each final evaluation group consisted of six rabbit eyes. In all instances both conjunctival sacs of a rabbit were instilled with 10 μl of either pitch volatiles or distilled water and one eye was subsequently irradiated with UV at 2.0×10^3 joule/m^2. No changes were observed in

irradiated or nonirradiated eyes given distilled water. In two eyes treated with pitch volatiles, injection of the lids, tearing, and slight mucous discharge were observed at 5 and 24 hours, but the eyes were normal thereafter. No corneal changes were observed. All six eyes treated with pitch volatiles and subsequently irradiated developed marked injection and edema of the lids, tearing, mucous discharge, and photophobia. These changes were pronounced at 5 and 24 hours after treatment, less pronounced at 48 hours, and had disappeared at 96 or 120 hours. Five hours after treatment the cornea appeared hazy and swollen with an opalescent ground-glass appearance and frequently surface pitting. A large central corneal ulcer, which stained with fluorescein, was present at 24 hours, smaller at 48 hours, present in only three eyes at 72 hours, and had disappeared by 96 hours. A granular ground-glass appearance and some swelling of the cornea remained for 24 to 48 hours after the ulceration had disappeared. All lesions appeared to heal completely and deeper structures such as the lens were not visibly damaged.

Two rabbits were killed 24 hours after treatment with pitch and subsequent irradiation. Histological examination of their eyes, stained with hematoxylin and eosin, showed marked congestion of the palpebral conjunctiva and extensive sloughing of the corneal epithelium with beginning superficial keratitis. No changes were observed in the iris, lens, or other ocular structures. Eyes treated with pitch alone showed edema and early cell necrosis of the corneal epithelium without other changes. Eyes treated with radiation alone were histologically normal.

These results indicate that exogenous photosensitizers such as coal-tar pitch components can cause phototoxic damage to the cornea and conjunctivae which can be assessed in animal models. The possibility of phototoxic eye damage should be borne in mind in evaluating the potential or actual effects of drugs or environmental contaminants.

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Stereospecific and Nonstereospecific Effects of (+)- and (−)-Morphine: Evidence for a New Class of Receptors?

Abstract. *The unnatural (+) enantiomer of morphine had minimal activity in three opiate assays in vitro: the rat brain homogenate binding assay, the electrically stimulated guinea pig ileum assay, and the inhibition of adenylate cyclase in neuroblastoma × glioma hybrid cell homogenates. When (+)-morphine was microinjected into the periaqueductal gray (a site known to mediate morphine analgesia) of drug-naive rats, there was only minimal analgesia, but the hyperresponsivity usually observed after microinjection of (−)-morphine occurred. Also, when (+)-morphine was microinjected into the midbrain reticular formation of drug-naive rats, rotation similar to that following microinjection of (−)-morphine occurred. These behaviors were not blocked by naloxone. Significantly, they typically occur in precipitated abstinence in morphine-dependent rats. These observations suggest that there are at least two classes of receptors, one stereospecific and blocked by naloxone and the other only weakly stereospecific and not blocked by naloxone, and that precipitated abstinence may be due, in part, to a selective blockade of receptors of the former class but not of the latter.*

The recent exciting discovery of the endorphins (*1*), whose potential neuromodulatory role in the central nervous system (CNS) (*2, 3*) has attracted widespread interest, was made possible by the earlier discovery of a class of CNS receptors which possessed stereospecific affinity for opiates and which were further characterized as being blocked by naloxone (*4*). In the studies reported here, we compared the (+) and (−) enantiomers of morphine in several parallel opiate assays in vitro and in vivo, and we demonstrated that there are two distinct classes of receptors that mediate morphine effects. Receptors of the first class, possessing a high degree of stereospecificity and being blocked by naloxone, mediate morphine analgesia. The endogenous ligands for these receptors are apparently the endorphins (*5*). Receptors of the second class, possessing a low degree of stereospecificity and not being blocked by naloxone, mediate the syndrome of hyperexcitability and explosive motor behavior seen after direct microinjection of morphine into certain CNS sites (*6*). This behavior is strikingly similar to some components of the precipitated abstinence syndrome, suggesting that these receptors may play a significant role in opiate dependence.

Unnatural (+)-morphine was synthesized from natural (−)-sinomenine as outlined in Fig. 1. Briefly, (−)-sinomenine was converted to the key intermediate (+)-dihydrocodeinone, and then to (+)-codeine. *O*-Demethylation of (+)-codeine gave 88 percent yield of pure (+)-morphine. The overall yield from the starting material was 25 to 27 percent. In earlier work Goto and Yamamoto (*7*) effected the conversion of (−)-sinomenine to (+)-morphine with a 3 percent overall yield. The (+)-morphine was chromatographically and spectroscopically indistinguishable from an authentic sample of the (−) enantiomer except for the sign of optical rotation.

The unnatural (+)-morphine, assayed in three opiate assay systems in vitro, had the following effects: it was 10,000-fold weaker than its natural (−) enantiomer in its ability to displace [^{3}H]dihydromorphine from binding sites in rat brain homogenates (Fig. 2A). In electrically stimulated guinea pig ileum, (+)-morphine did not inhibit contractions at a dose 100 times greater than the dose of (−)-morphine or of (−)-normorphine that is normally effective in inhibiting contractions. Furthermore, (+)-morphine did not antagonize the action of (−)-morphine or of (−)-normorphine in this assay (Fig. 2B). Finally, in the assay of adenylate cyclase activity in neuroblastoma × glioma hybrid cell homogenates, (+)-morphine had less than 1/1000