Histopathology of experimental ethambutol intoxication. SIMMONS LESSELL.

Ethambutol was administered to albino rats in their drinking water in doses of 105 to 2,500 mg. per kilogram per day for 18 to 102 days. Sixteen per cent developed bilateral lesions consisting of focal axonal swelling without demyelination, in their optic chiasms and the intracranial portions of their optic nerves.

Ethambutol (the dextro isomer of 2,2'- (ethylenediimino)d-l-butanol) is widely used for the treatment of pulmonary tuberculosis. Like all drugs it may produce undesirable side effects, the most notable being amblyopia. Instances of impaired vision were recognized shortly after the racemic form of the drug was introduced, but detailed case histories were not published until Carr and Henkind described toxic amblyopia in a man treated with the drug. Subsequently it was shown that although most of the therapeutic effect resided in the dextro isomer, the levo isomer possessed most of the toxicity. As a result ethambutol is now used only in the dextro form. Despite that, and limitation of dosage, cases of visual impairment due to ethambutol continue to be reported. In the only histopathological study of the brain and eye of such a patient there was demyelination in the optic chiasm. Studies of ethambutol intoxication in animals have also shown that lesions may occur in the optic chiasm. Stimulated by these observations, I investigated the effect of ethambutol intoxication in the anterior visual system of the albino rat.

Methods. CD strain, adult male rats of 175 to 413 grams were caged in pairs. Controls (23) received tap water ad libitum; 166 experimental rats received water containing between 0.5 and 2 per cent ethambutol. Control rats gained weight during the experiment, but failure to gain, or loss of weight, was the rule among the experimental rats. Of the experimental rats, 58 were discovered dead in their cages and were discarded. The 23 control rats and 104 experimental animals were killed at intervals of 18 to 102 days after the

Fig. 1. Cross section of anterior chiasm of intoxicated rat. There are many clear areas giving a multicystic appearance. (Luxol fast blue, cresyl violet; original magnification, ×100.)
Fig. 2. Cross section of intracranial segment of optic nerve of intoxicated rat showing multiple cystic lesions. (Luxol fast blue, cresyl violet; original magnification, ×100.)

drug was started. Their daily dose (calculated from the amount of water consumed) averaged between 105 and 2,500 mg per kilogram. Seventeen experimental rats and six control rats were perfused with phosphate-buffered glutaraldehyde through the heart after being anesthetized with an intraperitoneal injection of pentobarbital. The other rats were killed with pentobarbital but were not perfused. Both eyes, both optic nerves, the chiasm, and brain were removed from each rat. Samples from the retinas, nerves, and chiasms of the perfused rats were fixed in phosphate-buffered glutaraldehyde, embedded in Epon, cut in 1 μ cross sections, and stained with toluidine blue. Tissues from the nonperfused rats were fixed in 10 per cent neutral formalin, trimmed, and embedded in paraffin to provide a central horizontal section of each eye, a longitudinal section of the orbital portion of one nerve, and transverse sections of the intracranial portions of the nerves, the orbital portions of one nerve, and the optic chiasm. The chiasm and nerves were studied subserially from the globe to the optic tracts, in 7 μ sections stained with luxol fast blue and cresyl violet.

Four experimental and two control rats were used for histochemical studies. Their optic nerves and chiasms were quickly removed, frozen in dry ice, and transferred to a cryostat. Unfixed, frozen sections were cut at 17 μ and incubated in substrates suitable to demonstrate activity of the following enzymes: alkaline phosphatase, acid phosphatase, lactic acid dehydrogenase, glucose-6-phosphate dehydrogenase, and monoamine oxidase.

**Results.** By the time they were sacrificed, most experimental rats showed alopecia, epistaxis, lethargy, ataxia, and tremulousness. Many were abnormally docile, but it could not be established whether they were blind. The tissues of all the rats looked grossly normal when examined with the dissecting microscope. No abnormalities were
Fig. 3. Cross sections of anterior chiasm of control rat (A) and intoxicated rat (B) showing dilated axones in the latter. (Toluidine blue, 1 μ sections; original magnification, ×400.)
seen in the sections that were incubated to show enzymes. The location and intensity of enzyme activity was identical in control and experimental rats. Patterns of phosphatases and dehydrogenases in the optic nerves were identical to those reported previously in normal albino rats.6

Sixteen experimental rats had stereotyped lesions which were bilateral (but not necessarily symmetrical) and involved the chiasm and adjacent parts of the optic nerves (Figs. 1 to 3). The lesions consisted of scattered or clustered vacuoles, which represented dilated axones. The myelin sheaths of dilated axones were slightly thinned, but demyelination was not observed. Both crossing and noncrossing axones in the chiasm were involved, and the axones appeared to be focally enlarged (Fig. 4). The glial cells, blood vessels, and meninges were normal. No lesions were found in the retinas, optic nerve heads, or the intraorbital segments of the optic nerves.

Comment. This investigation has demonstrated that lesions can be produced in the optic chiasms and optic nerves of rats by feeding them huge doses of ethambutol. The lesions cannot be ascribed to the inanition that occurred in the intoxicated rats because such lesions were never found in malnourished rats studied by the same techniques in this laboratory as part of an investigation of experimental cyanide intoxication. Comparison with previously reported studies of intoxication with ethambutol shows that there are both similarities to and differences from those results in the present study.

In one set of studies,5, 6 monkeys were intoxicated with the racemate in dosages of 400 to 1,600 mg. per kilogram per day, or the D-form in doses of 25 to 1,600 mg. per kilogram per day for up to 26 weeks. The racemate appeared to be at least twice as toxic as the D-form but the signs of intoxication and the type of lesions produced in the nervous system were similar.
They differed mainly in severity. Some monkeys behaved as if blind, but their fundi looked normal and the histopathological appearance of their retinas was probably normal. The first lesions to appear with graded doses of ethambutol were in the optic chiasm, or the optic chiasm and optic tracts. In severe lesions there was central necrosis with a macrophage response. When the optic nerves were involved, the lesions were located predominantly in their middle or distal portions. Ethambutol intoxication was also studied in rats and dogs. Blindness was not described, and all of the retinas were normal when examined microscopically; however, virtually all of the animals had lesions in their optic nerves or optic tracts that resembled those found in the intoxicated monkeys. No description of the optic chiasm was provided. A third group of investigators fed ethambutol to seven rabbits for periods of 8 to 232 days. Two rabbits developed "mild demyelination and axonal fragmentation" in their optic nerves.

These studies have established in several species that the anterior visual pathway is vulnerable to the toxic effects of ethambutol. An extraordinary feature of experimental ethambutol intoxication is the predilection for lesions to develop in the chiasm. These lesions are primary, and not secondary to axonal lesions elsewhere. No other drugs have been shown to produce chiasmal lesions in experimental animals, and evidence of primary chiasmal involvement is scarce in humans with toxic amblyopia. There have been patients in whom it appeared that isoniazid, chloroquine, ethchlorvynol and pheniprazine produced signs and symptoms suggestive of involvement of the chiasm. Several patients have been reported who developed bitemporal hemianopia while being treated with ethambutol and a chiasmal lesion has been reported at autopsy in a patient presumed to be intoxicated with ethambutol. No other instances of drug-induced chiasmal damage have been demonstrated histopathologically in a human insofar as I know.

Most patients with ethambutol-induced amblyopia recover when the drug is discontinued. A lesion of the type found in the present study, consisting of axonal swelling, should be reversible, and might be the early lesion that occurs in most patients with ethambutol intoxication. The necrotic lesions described in other experimental studies may represent the end-stage of prolonged intoxication. Such lesions would not be reversible, and lesions of that type are probably present in those patients who have been reported to have irreversible, ethambutol-induced amblyopia.

The reason for the unusual distribution of the lesions in ethambutol intoxication is unknown. One possibility is that the drug becomes deposited selectively in the optic chiasm and adjacent portions of the nerves. A second possibility is that variations in vasculature determine the differential susceptibility of different parts of the anterior visual pathway. A third possible explanation is that the involved structures are bathed in cerebrospinal fluid, whereas the uninvolved segments of the optic nerves have limited contact with cerebrospinal fluid. It is also possible that regional variations in the glial population or their metabolic characteristics are a determinant. Finally, it is conceivable that variations in the activity of enzymes or in the concentration of metals along the course of the anterior visual pathway could produce regional variation in susceptibility to the toxic effects of ethambutol. My future investigations will be aimed at defining the ultrastructure of the lesions and attempting to explain their pathogenesis and distribution.

Joanna Smith, Rozanne Richman, Marjorie Parmenter, Miller Lessell and Jason Lessell did all the technical work. Jason Lessell translated some of the foreign literature.

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