Leukocyte counts. Mean leukocyte counts for mice infected via the anterior chamber or by corneal scratch during the 9-day period after cyclophosphamide are plotted in Fig. 3. All animals were leukopenic and had \(<700\) leukocytes/mm\(^3\) of blood on the day of challenge. Cell numbers were within the normal range by day 7.

Discussion. In man, corneal infections produced by \(P.\ aeruginosa\) usually evolve following injury to that organ and may result in blindness. Infections also are observed which are not due to direct corneal injury but occur indirectly as a result of severe immunological debilitation. Rosenoff et al.\(^7\) reported severe infection of the lid with \(P.\ aeruginosa\) recovered from the eye exudate in a patient with carcinoma who had received a combination of chemotherapeutic drugs. Additionally, Ziegler et al.\(^3\) have observed that 90% of the animals died of overwhelming bacteremia (24 hr.) and over half of these displayed conjunctivitis when infected with \(Pseudomonas\) (in their drinking water) followed 2 days later by a single i.v. injection of nitrogen mustard.

The usual response of Swiss-Webster mice challenged with \(Pseudomonas\) intraocularly is the development of a purulent exudate (composed predominantly of PMN's), with corneal opacity visually observable 18 to 24 hr. after bacterial challenge.\(^2\)\(^,\)\(^3\) This infection spontaneously resolves within about 4 weeks in mice,\(^2\)\(^,\)\(^3\) although we previously have reported a quite dissimilar response for an inbred mouse strain.\(^5\) Pretreatment of Swiss-Webster mice with a myelosuppressive agent such as cyclophosphamide produces a marked decrease in circulating leukocytes. Since the combined activity of serum opsonins and granulocytes is a primary defense in normal hosts against \(P.\ aeruginosa\) infection,\(^8\) a 10-fold decrease in natural resistance to \(Pseudomonas\) produced by a single i.p. injection of cyclophosphamide compromised the treated mice to the extent that septicemia and death resulted (particularly at higher dilutions of the organism). Corneal damage was observed both histologically and ultrastructurally in surviving mice. The results with cyclophosphamide described here were unexpected since similar studies with methotrexate did not result in the potentiation of a systemic infection despite a similar transient leukopenia.\(^10\) The preliminary results described in this report have potential clinical significance for those patients undergoing antineoplastic chemotherapy. An accidental infection of the eye in neutropenic patients could conceivably result in an overwhelming systemic infection.

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Key words: \(Pseudomonas aeruginosa\), cornea, proteoglycan, collagen, neutrophils, cyclophosphamide, mice.

REFERENCES


Embryopathic effect of ophthalmic EDTA.

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Studies on the potential teratogenic effect of topically applied EDTA (0.1% and 3%) were undertaken because of its proven teratogenic effects when administered systemically and because of its wide use as an ophthalmic drug. Although
no teratogenic effect was found for either 0.1% or 3% solution of EDTA, 3% EDTA applied topically to the eye six times a day has a significant embryopathic effect, with only 30% of the progeny remaining normal.

The fact that ophthalmic medications can produce teratogenic effects when topically applied to the eye in doses equivalent to those used clinically has stimulated interest in the potential teratogenic effect of other commonly used ophthalmic drugs.

Ethylenediamine tetraacetate (EDTA, Edathamil) is a chelating agent with a high affinity for many metals. Increased use of metal-binding substances in medicine has stimulated interest in the potential toxic effect of EDTA and related chelating compounds. Early evidence of chelate toxicity appeared in 1956, shortly after synthetic chelating compounds became available. Injection of EDTA into pregnant rats resulted in congenital malformations in the young. Since it was known that this chelating agent binds calcium, the authors suggested that impairment of fetal development might be due to maternal hypocalcemia. Recently, it has been shown that ingestion of EDTA by female rats during pregnancy impaired reproduction and resulted in congenitally malformed young. When EDTA was fed from days 6 to 21 of gestation, all the full-term young had gross congenital malformations. These effects were prevented by simultaneous supplementation with 1,000 p.p.m. of dietary zinc, therefore suggesting that the congenital anomalies caused by EDTA were due specifically to zinc deficiency.

Studies on the potential teratogenic effect of topically applied EDTA were undertaken because of its proven teratogenic effects when administered systemically and because of its wide use as an ophthalmic drug. The purpose of this study was to evaluate carefully the teratogenic effect of EDTA in rabbit embryos when topically applied to maternal eyes.

Materials and methods. Eight adult female albino rabbits were each mated twice with the same male rabbit. Day of conception was determined by the presence of sperm on the vaginal smear. The pregnant rabbits were randomly divided into two groups. One group was treated with 0.1% EDTA solution and the other group with 3% EDTA solution, both freshly prepared from a 5% solution of the disodium salt of EDTA solution (Sigma Chemical Co., St. Louis, Mo.). Both groups received 2 drops of the medication in each eye six times a day from the sixth to the eighteenth day of gestation. All rabbits were sacrificed on day 29 of gestation; the fetuses were removed by cesarean section and carefully examined for external malformations, then sacrificed by immersion in formaldehyde and ethanol solution. In addition, serial histologic sectioning and skeletal examination with alizarin red-S were done in all cases, whether or not malformations were suspected.

Results. The effects of topically applied 0.1% and 3% EDTA solution to both eyes of pregnant rabbits from the sixth to the eighteenth day of gestation are summarized in Table I. Although no teratogenic effect was found for either 0.1% or 3%, the higher embryocidal effect of EDTA at 3% concentration was too high not to be considered due to the effect of 3% EDTA. The fraction of progeny which remained normal was 89% for the 0.1% group and 30% for the 3% EDTA group.

Discussion. Among the factors which determine the teratogenicity of a particular compound are (1) the dose of the teratogen or drug, (2) the duration of exposure, (3) the effect of absorption and metabolism, and (4) the effect of gestational age.

The lack of teratogenic effect of EDTA when given topically to the eye, in contrast to its teratogenic effect when given by mouth or injection, can be explained by the dose of the drug and the effect of absorption. One drop of 0.1% or 3% EDTA six times a day, if fully absorbed by the eye, compounds to about 0.3 or 9 mg. a day. In contrast, the diets containing EDTA were prepared by adding either 2 or 3 gm. of NaEDTA to 100 gm. of the control ration. In addition, EDTA is known not to penetrate the corneal epithelium.

Teratogens can be classified into two main groups: (1) those which appear capable of inducing effects at any doses and (2) those for which minimum doses are required before embryopathic effects can be observed. EDTA exhibits a "teratogenic zone" only at high doses and is best classified as the second type of teratogen.

In addition to teratogenesis, fetal death may result either from direct toxic effects on the fetus or from pharmacogenic or toxic effects of the drug on the mother. Also, it is impossible to determine whether or not those embryos which were resorbed might also have been malformed had they survived in utero for a longer period of time. Therefore it is difficult to identify clearly each kind of embryopathic effect at the time pregnancy is terminated. If one then considers the effect of the drug on the embryo-fetal system,
namely the number or fraction of progeny which remained normal, 3% EDTA applied topically to the eye six times a day has a significant embryopathic effect, with only 30% of the progeny remaining normal. No teratogenic or embryopathic effects have been observed. Nevertheless, the implication of this study merits careful consideration if this compound (EDTA) is to be used in pregnancy, especially during the first trimester.

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REFERENCES


The disulfide form but not the sulphydryl form of a boron hydride compound was found to be cataractogenic. Apparently this compound attaches to the sulphydryl group of Na-K ATPase in the lens epithelium inactivating this crucial enzyme. The consequence is that a defect in the cation pump activity arises, leading to a rapid influx of Na ions and loss of K ions and marked increase in hydration. These changes are thought to lead to opacification.

Boron hydride compounds have been investigated because of their possible usefulness in neutron therapy of brain tumors. To increase the uptake of the boron hydride anions into tumor cells, a sulphydryl moiety was incorporated into the structure. These compounds were taken up by tumor cells, but their toxicity was increased. Two interesting compounds were the sulphydryl (monomer) and disulfide (dimer) forms of a boron hydride compound (Fig. 1). The disulfide form caused an acute form of cataract in mice, but the sulphydryl form did not. This report deals with studies attempting to determine the cataractogenic effect of the boron hydride compound.

Material and methods. Cesium salt of the monomer (Cs_2BnHnS) and of the dimer, or the disulfide form (Cs_2BnHnS)_2, as well as their tritiated forms were furnished by Dr. Hideo Terao, Neurosurgical Laboratories, Massachusetts General Hospital, Boston.

A description of the incubation and chemical procedures used in these experiments has been reported previously. The medium employed was the TC-199, bicarbonate and glucose mixture as described previously. The incubations were for an overnight period unless otherwise specified.

Na-K ATPase was prepared from the capsule epithelium of 100 calf lenses. The tissue was homogenized in 10 volumes of 0.01M Tris buffer, pH 7.2. The homogenate was centrifuged, and the precipitate recovered was resuspended in Tris buffer and centrifuged. The washed precipitate was suspended in one third of the original volume of the buffer, and this suspension served as the enzyme source. Protein was determined by the Lowry method on the suspension. Na-K ATPase activity was determined as previously described.