that a fibrinolytic agent (urokinase) and an inhibitor (mingin), both normally present in the human body, are greatly influenced in their effects by the presence of EACA. While higher concentrations of EACA inhibit the fibrinolytic activity of urokinase small concentrations enhance the effect. EACA greatly potentiates the inhibitory effect of mingin on urokinase. This was observed even at low concentrations where EACA normally enhances urokinase induced fibrinolysis.

The results obtained are of importance, because administration of EACA as an antifibrinolytic agent is gaining widespread use. The potentiating effect of even small amounts of EACA on inhibition of urokinase by mingin is especially significant, since this inhibitor is normally excreted in human urine(6). The excretion increases considerably during pregnancy. An average excretion per 24 hours of around 15,000 units (one inhibitor unit equals 1 μg trypsin) is reached during the third trimester followed by a steep fall after parturition(7). Large increases in excretion occur also after administration of ACTH or cortisone as well as during various forms of stress(8). The concentration of mingin per ml urine encountered in such cases is in the same range as used in the experiments described above. Hence, the potentiating effect of EACA on the inhibition by mingin should be taken into consideration when EACA is administered to patients.

Summary. The antifibrinolytic activity (assayed against urokinase) of the trypsin inhibitor (mingin) isolated from human urine is greatly potentiated by ϵ-aminocaproic acid. Potentiation of inhibition occurs even at concentrations of EACA where normally the fibrinolytic activity of urokinase is enhanced.

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Abnormalities in Chick Embryos Following Thalidomide and Other Insoluble Compounds in the Amniotic Cavity.* (28241)

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Thalidomide (N-pthaloyl glutamimide) has been implicated in a variety of congenital defects of infants whose mothers were given the drug during early pregnancy(1,2,3,4,5,6). The most common defects were amelia, phocomelia, defects of the digits and alimentary abnormalities. Other defects included hemangiomas of the nose and upper lip, dysplasia of the external ears or eyes and defects of the heart and genitourinary system. Since the chick embryo has been used in this laboratory to study the teratogenic activities of viruses(7,8,9,10) and antitumor drugs(11), it seemed that these techniques might be useful to study the teratogenic activity of thalidomide. Introduction of thalidomide into the amniotic cavity of early embryos resulted in encephalocele and eye or eyelid defects in a significant percentage of the treated embryos. However, these defects in no way resembled the thalidomide-induced defects of the human. The thalidomide was not readily soluble in the diluents used and some of it was observed to remain undissolved for several days in the amniotic cavity of the chick embryos. Further investigation showed that other insoluble compounds also produced similar abnormalities following amniotic inocu-

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lations. In the following report are detailed results of these studies.

Methods and materials. Thalidomide was supplied by the Wm. S. Merrell Co., Cincinnati, Ohio. Since it was highly insoluble in ordinary diluents, it was ground in a Ten Broeck tissue grinder and suspended in saline or normal allantoic fluid. Inoculations were made into White Leghorn eggs incubated in a forced draft incubator at 100°F. For inoculations into the amnion, the shells were prepared with windows(8). Control embryos, from the same incubation setting, were inoculated with saline or normal allantoic fluid by the same route as the experimental group. After inoculation the eggs were observed daily and the dead ones were examined for defects. The surviving embryos were harvested on the 16th to the 18th day of incubation. A group of "control compounds" to be tested by inoculation into the amnion were selected for their lack of solubility in water or saline and for their relative lack of toxicity in biological systems. These were fine sand, ground glass, colloidal alumina (Baymal) and attapulgus clay (a mixture of metal oxides), also colloidal.

Results. Amniotic inoculations of 264 embryos of 3 days incubation with single doses of 1 or 2 mg of thalidomide consistently produced a significant percentage of embryos with encephalocele and/or eye or eyelid defects (Fig. 1, 2). In the experimental group 7% showed limb bud defects and 9% showed ectopic viscera. In 123 embryos inoculated with saline 3% showed limb bud defects, 2% showed ectopic viscera but none showed encephalocele or eyelid defects. The limb bud defects, characterized by absence or retardation, occurred chiefly among embryos dying early in development, whereas the encephalocele and eyelid defects were rarely observed in embryos of less than 10 days incubation. The encephalocele usually occurred over the region of the frontal suture, either in the midline or to the left. In some cases it consisted of complete absence of the bone and skin over the area with extrusion of the cerebral hemispheres. In milder cases the skin was intact but devoid of feather follicles. The eyelid defects ranged from small irregularities of lid development to severe microblepharon or even absence of the lids. In many cases the eyeball protruded beyond the socket. In some,

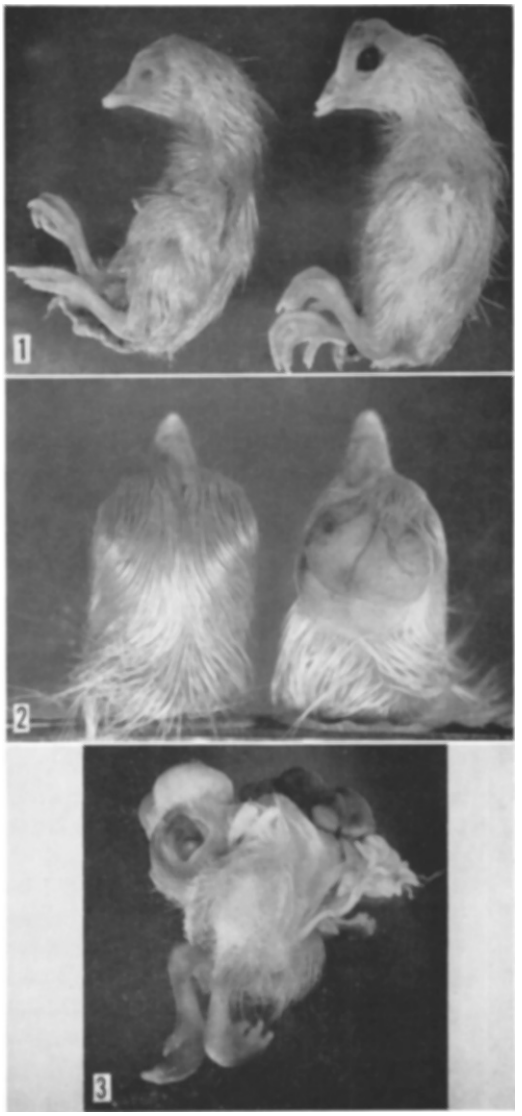

FIG. 1. Chick embryos of 16 days incubation. Left shows normal embryo from saline control group. Right shows embryo with encephalocele, eyelid defect and short upper beak following 1 mg thalidomide at 3 days incubation.

FIG. 2. Chick embryos of 16 days incubation. Left shows normal embryo from saline control group. Right shows embryo with encephalocele following 1 mg thalidomide at 3 days incubation.

FIG. 3. Chick embryo of 15 days incubation shows encephalocele, eyelid defect, short upper beak, ectopic viscera and twisting of body axis following colloidal alumina at 5 days incubation.

TABLE I. Effect of Thalidomide Inoculated into Amniotic Cavity of Chick Embryos of Various Incubation Ages.

Incubation days at inoculation	Thalidomide (mg/egg)	Total eggs inoc	Survived to 12th day or more of incubation	No. showing encephalocele or eyelid defects (No.)	(%)
3	1 or 2	264	124	45	(36)
4	2	20	5	5	(100)
5	2	28	20	15	(75)
6	2	28	19	4	(21)
7	4	22	15	1	(6)

TABLE II. Effect of Various Insoluble Compounds Inoculated into Amniotic Cavity of Chick Embryos.

Compound	Incubation days at inoculation	(mg/egg)	Total eggs inoc	Survived to 12th day or more of incubation	No. showing encephalocele or eyelid defects (No.)	(%)
Sand	3	2.0-1.0	22	6	3	(50)
Alumina	5	.1- .01	47	24	17	(70)
Attapulgus clay	5	1.0- .001	107	56	26	(46)
Glass	5	2.0-1.0	26	19	15	(79)
Saline	5		100	63	0	

the eye was covered with a cyst filled with clear fluid and in several a strand of tissue, possibly amnion was firmly attached to the eye. In 3 embryos out of 30, only the right eye was affected, in 6 the defect was bilateral and in 21 only the left eye was affected. Frequently observed in embryos showing either eyelid defects or encephalocele were crossed beak and/or short upper beak. Table I shows the incidence of embryos with these characteristic defects according to age of inoculation of the thalidomide.

Table II shows that all 4 of the selected insoluble control compounds produced encephalocele and eyelid defects exactly like those following thalidomide. Short upper beak and crossed beak were also observed with significant frequency except following sand where the number of embryos was too small to be of significance. In addition, alumina and attapulgus clay, particularly in the higher dosages, produced severe twisting and distortion of the body axis and appendages (Fig. 3) although these appendages appeared normal in general development. About a third of these embryos surviving 12 days or more showed ectopic viscera or failure of ventral closure. This defect was even more frequent in embryos dying at earlier stages. The eye defects were bilateral in most of the severely twisted embryos, but the unilateral eye defects occurred chiefly on the left, as with the thalidomide.

Discussion. It seems unlikely that all the compounds tested have any biological activity in common and it is presumed that the defects occurred because of the presence of extraneous particulate matter in contact with the developing embryo. The fact that the amount necessary for production of the defects was much less with the colloidal substances than with thalidomide may have been due to the greater chance for contact with the body coverings when the material was widely dispersed. This might also explain the additional defect of distortion of body parts and increased incidence of ectopic viscera seen with the colloidal substances. The increased incidence of left eye defect might logically be assumed to result from adherence of the particles as the embryo, which lies on its left side, comes in contact with the amnion. This is in accordance with the fact that the embryo begins to move and turn freely in the amniotic sac on the fifth day and the lids and nictitating membrane begin differentiating about the seventh day. The encephalocele and eye-eyelid defects seem to be

part of the same defect, an absence of skin and bone with resulting exposure of the underlying structures. The structures which were susceptible to damage may be considered to be directly exposed to contact with the insoluble compound if one assumes that absence of the skin in turn exposes the mesenchyme of the head from which the membranous frontal bone forms between the tenth and twelfth day. Both the eyelids and the frontal bone are in beginning stages of differentiation during the time of exposure. The mechanism by which the defects occur is not known. It may be due to interference with further development of the structures or to actual destruction of the tissues already developed, possibly through irritation or abrasive action. Whether there is any relationship of etiology between these defects and similar defects which are encountered in the human is not known.

With the increasing interest in screening of drugs for possible teratogenic effects it is necessary to consider carefully the physical characteristics of the drug to be tested as this factor influences the experimental testing system utilized.

Summary. Thalidomide produced encephalocele, eyelid defects and beak defects in a significant percentage of chick embryos inoculated into the amnion with the drug at 3 through 6 days incubation. No defects of this type were observed in control embryos inoculated with saline. However 4 other selected compounds which, like the thalidomide, remained in an undissolved state in the amniotic cavity after inoculation, produced the same types of defects. These compounds were sand, ground glass, colloidal alumina and colloidal attapulgus clay. In addition the last 2 produced severe twisting and distortion of the axis and appendages of the embryos, with increased incidence of ectopic viscera.

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Collection of Monkey Semen by Electroejaculation.* (28242)

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Electroejaculation has been used for semen collection in several species, including the bull, boar, dog, ram, fox, chinchilla and guinea pig (1,2,3). This approach has not, to our knowledge, been successful in the primate, notwithstanding the numerous practical applications of such a method in monkey breeding and reproductive research. Recently, this relatively unexplored area of human physiology has been emphasized and unsuccessful attempts at electroejaculation reported(4). The method reported here provides a simple technique for electroejaculation in the macaque, which is not associated with a generalized convulsive reaction and has yielded almost consistently satisfactory results.

Method. Adult male macaques were restrained either by 3 assistants or by one assistant using a choke chain restraint within the cage. Gentle traction was applied to the glans penis. A strip of aluminum foil 2 cm

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