Summary. 1) Mice were capable of being protected against a number of isolates of *C. neoformans* by passive immunization with antiserum. This protection was limited to the immunization period and was type-specific. 2) *In vivo* and *in vitro* phagocytosis of *Cryptococcus* cells has been demonstrated. When large and small capsule variants were studied separately, the former were practically resistant and the latter relatively susceptible to ingestion by mouse PMN. 3) The probable mechanism for the protection achieved has been discussed and a possible use for antiserum has been proposed.

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Delayed Hypersensitivity *in vitro*. III. Effect of Cortisone on Cytotoxicity of Mumps Viral Antigen.* (24124)

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Development of delayed hypersensitivity is a characteristic response of the human host in a number of infectious diseases, including mumps and tuberculosis(1-7). Recent studies have demonstrated development of delayed hypersensitivity in experimental mumps virus infections in guinea pigs and its manifestation *in vitro* by a cytotoxic effect of mumps virus antigen on cells of tissue cultures prepared from spleens of infected animals similar to that seen in tuberculosis(8). Successful treatment of complications of mumps virus infections(9-15), particularly mumps orchitis, with corticosteroids, combined with evidence that corticosteroids will alter delayed hypersensitivity response to tuberculin *in vivo*(16-20) and suppress cytotoxic effect of tuberculin *in vitro*(21), suggest a possible relationship between hypersensitivity to mumps viral antigen and therapeutic action of corticosteroids in mumps virus infections. It was our purpose to study the effect of cortisone on *in vitro* cytotoxicity of mumps virus antigen for hypersensitive cells. A tissue culture system which permits observation and evaluation of the effect of cortisone on a cellular level independent of vascular components and nonspecific tissue responses was used.

Materials and methods. The egg-adapted strain of mumps virus used was obtained from Dr. John F. Enders. Preparation of stock virus and antigen has been described(8). Guinea pigs, weighing at least 250 g, were infected with mumps virus by 2 routes. Following ether anesthesia, the eye was immobilized and 0.03 ml of virus-containing fluid was injected into the anterior chamber with tuberculin syringe; 0.1 ml of virus fluid was also dropped into nasal passages of the animal. Three to 4 weeks after infection the guinea pigs were skin tested with mumps virus antigen prepared as previously described(8). The tissue culture method was the same as outlined by Gangarosa *et al.*(4). The experimental animal was sacrificed, the spleen removed under sterile conditions, and the tissue minced in a small test tube with Hanks' balanced salt solution (BSS)(22). Fragments about 1 mm^3 were placed on a rooster plasma coagulum in T-9 flasks (Konte Glass Co., Vineland, N. J.). Two ml of medium were added to each flask. The flasks were sealed with a silicone stopper and incubated at 37°C. The medium consisted of 60% BSS, 30%

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beef serum ultrafiltrate, 10% heated guinea pig serum, 0.05 to 0.1 mg/ml soybean trypsin inhibitor (General Biochemicals, Chagrin Falls, O.), 150 units/ml of penicillin, and 100 γ/ml of streptomycin. Approximately 45 fragments were included in each test group. Cortisone acetate (Merck and Co.), 25 γ/ml, was added to the media prior to placing it in T-flasks, approximately 24 hours before addition of mumps virus antigen.

Results. The course of ocular infections was similar to those previously reported(8). Twenty-one guinea pigs were inoculated with mumps virus and development of delayed hypersensitivity was determined by intradermal injection of mumps skin test antigen. Fifteen of the 21 guinea pigs developed positive skin tests with areas of induration measuring 3-10 mm in diameter. No control animals developed positive skin tests.

Our data confirm the observations reported earlier(8) of the *in vitro* cytotoxic reaction between mumps virus antigen and hypersensitive cells. There was some difference, however, in manifestation of toxicity in that a greater inhibition of migration of macrophages was noted. Not only were there fewer cells migrating from fragments exposed to mumps virus antigen (Fig. 1), but these cells also evidenced progressive cellular destruction (Fig. 3) in comparison to controls (Fig. 2), as had been observed previously(8).

In contrast, cells migrating from fragments

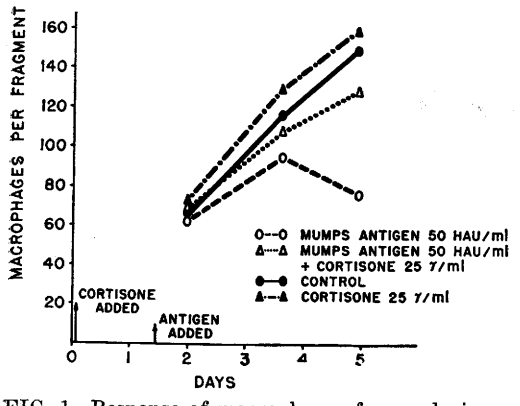

FIG. 1. Response of macrophages from splenic explants of a mumps skin test positive guinea pig. Cortisone was added at time of planting, mumps antigen at 36 hr. HAU = Hemagglutinating units of virus. Graph is that of a typical experiment and representative of 10 experiments.

TABLE I. Effect of Mumps Viral Antigen and/or Cortisone on Migration of Macrophages.

Exp. No.	Control	Cortisone control	Cortisone + mumps antigen	Mumps antigen
3-F	152*	160	129	75
H	105	100	95	56
I	120	135	130	44
K	272	299	280	158
M	105	112	98	51
L	110	118	100	60

* Avg No. of macrophages/fragment recorded on Day 5 (*i.e.*, 96 hr after addition of mumps antigen).

in flasks to which cortisone was added prior to addition of mumps antigen were protected from the cytotoxic action of viral antigen. This protection was noted both quantitatively (Fig. 1, Table I) and qualitatively (Fig. 1-4). These cells did not show inhibition of migration, or swelling and vacuolization, clumping, disruption of cell membrane and giant cell formation leading to eventual degeneration seen in cultures without cortisone. Protection, however, was not complete, since mild toxicity was noted in the cortisone group. However, this toxic reaction was slight in comparison with the cultures exposed to mumps antigen alone (Table I), and, since twice the minimal amount of virus required to produce the cytotoxic effect was used in these experiments, it is unlikely that the stimulating action of cortisone on macrophage migration masked any cytotoxic effect of the viral antigen.

Discussion. Corticosteroids have been employed in clinical medicine to suppress inflammatory response associated with infectious processes, thus diminishing tissue reaction. On this basis a number of workers have treated the complications of mumps virus infections, particularly mumps orchitis, with these drugs. In most reports there has been dramatic relief of the symptomatology within 24 hours (9-15). No data are available which suggest the nature of the processes involved, *i.e.*, whether the action is a nonspecific, anti-inflammatory effect or whether the corticosteroids alter basic interaction of host and parasite which results in tissue destruction.

It has been postulated(8) that the cell destructive potential of mumps virus is a func-

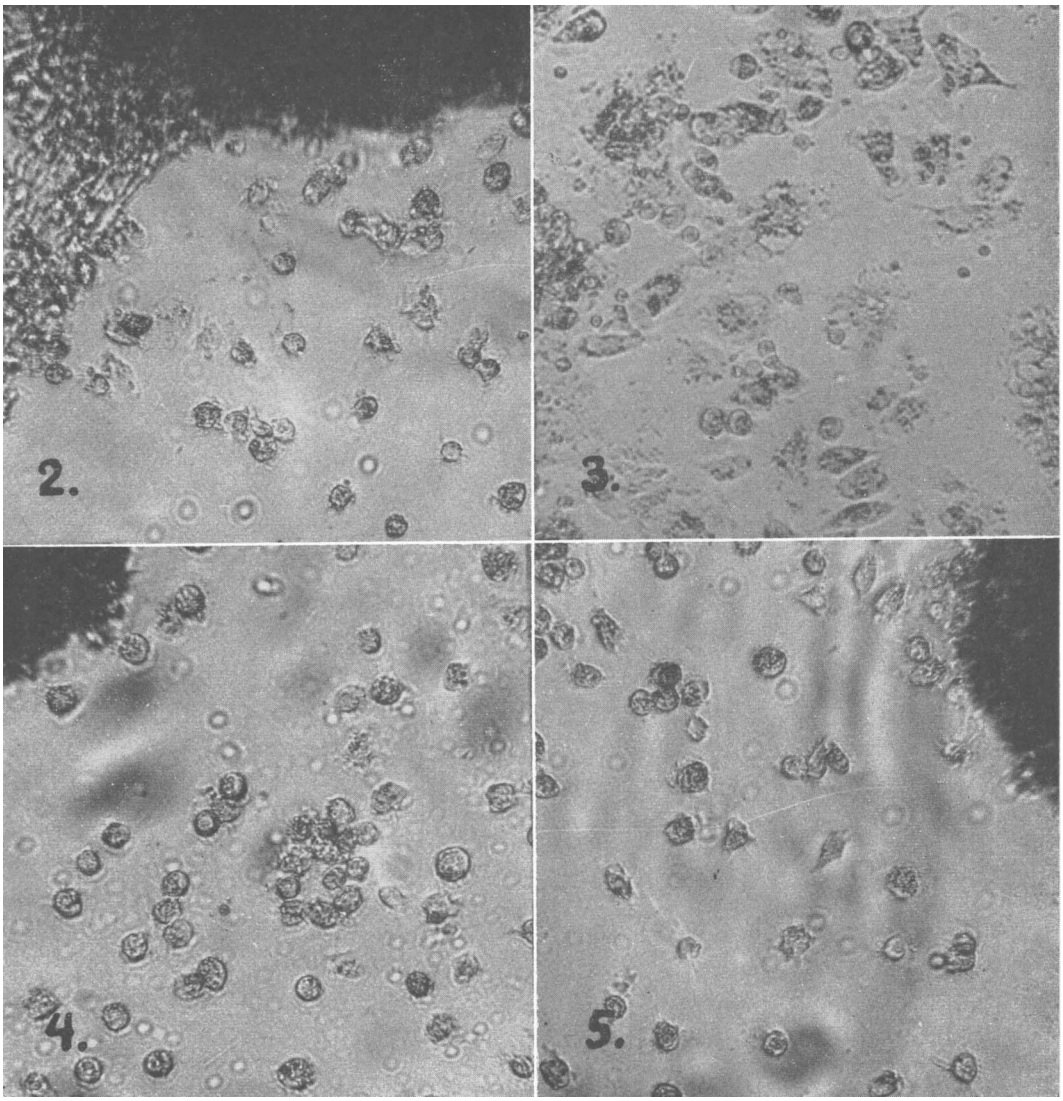

FIG. 2. Photomicrograph of spleen from skin-test positive guinea pig grown as normal control at 120 hr. ×100.

FIG. 3. Photomicrograph of spleen from skin-test positive guinea pig grown in presence of mumps virus antigen 72 hr after addition of antigen. ×100.

FIG. 4. Photomicrograph of spleen from skin-test positive guinea pig grown in presence of mumps virus antigen and cortisone acetate 72 hr after addition of antigen and 120 hr after addition of cortisone. ×100.

FIG. 5. Photomicrograph of spleen from skin-test positive guinea pig grown in presence of cortisone acetate 120 hr after addition of cortisone. ×100.

Note: All tissue cultures are 5 days old.

tion of 2 processes: (a) primary destruction of cells through direct cytolysis by actively multiplying virus; and (b) a secondary cytotoxic effect of mumps virus antigen on hypersensitive cells. Assuming this hypothesis to be true, then the data presented, within the limitation of the *in vitro* experimental situation, suggest a rationale for treatment of the complications of mumps virus infections in man with corticosteroids, on the basis that they block the hypersensitive component of the inflammatory reaction.

The effect of the corticosteroid compounds on the delayed hypersensitive state of tuberculosis has been well documented. *In vivo* studies(16-18) have demonstrated the ability of these compounds to alter the tuberculin reaction and Vollmer(20) noted reversal of tuberculin skin tests following treatment with steroids. Leahy and Morgan(21) reported suppression of the *in vitro* cellular reactivity to tuberculoprotein, indicating that the compounds have a direct action at the cellular level.

The observations presented here provide further evidence of the similarity of the delayed hypersensitive state in tuberculosis and mumps by demonstrating a protective action of corticosteroids on cytotoxicity of mumps viral antigen for hypersensitive cells *in vitro*.

Summary. Experimental mumps virus infections in guinea pigs are characterized *in vivo* by a positive delayed hypersensitive skin test reaction to viral antigen and *in vitro* by a cytotoxic effect of mumps virus antigen on cells from tissues cultivated from mumps-infected animals. Cortisone protects against this *in vitro* cytotoxicity of mumps virus.

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Increased Follicle-Stimulating Activity in the Plasma of Ovariectomized Rats.* (24125)

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Several investigators, using the technic of parabiosis, have been able to demonstrate that bilateral castration results in increased gonadotrophic activity in blood plasma of male and female rats(1). Emery(2) was able to show this increased activity by injection of castrate male serum into immature rats, and also reported on the basis of a few preliminary tests that serum from castrate, multiparous female rats showed gonadotrophic activity. Donor animals used were rats sacrificed over a 2 year period for various other purposes, and no attempt was made to standardize the post-operative period. The present study, in which immature hypophysectomized female rats

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