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Studies of Adenovirus Type III Infection Treated with Methisazone and Trifluorothymidine* (32862)

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Adenovirus infection of man is common and some adenoviruses have been implicated as causative factors of cancer in animals. Some types of adenovirus produce sufficiently mild infections, however, so that volunteer studies with these agents appear relatively simple and risk free (1,2).

The testing of drugs on randomly occurring diseases is difficult, since a definite clinical diagnosis may not be possible until the disease is past. In addition, the variability of randomly occurring cases, except in very large epidemics, would require that very large numbers of people be tested with any new drug which is to be evaluated in order for such studies to be meaningful. Volunteer studies of induced infection appeared safer and more practical than the study of random cases as a method of drug evaluation, and adenovirus type III, passed only in human amnion cells, and isolated from a very mild local infection seemed an ideal agent to provide this test system.

Two drugs possess *in vitro* activity against adenovirus and seem reasonably safe for use in man. Methisazone (*N*-methylisatin-*B*-thiosemicarbazone) is of proven value in a variety of pox virus infections of man, clearly preventing smallpox in contacts and probably providing therapeutic benefit in the treatment of vaccinia gangrenosa (3–13). Recent studies of this drug in tissue culture indicate that it has some *in vitro* effect in suppressing adenovirus multiplication (14), and a broad experience with thousands of subjects provides detailed information as to its safety and toxicity (3–6). Trifluorothymidine (F_3TdR) is a potent and nontoxic antimetabolite of proven benefit in the experimental and clinical treatment of herpes simplex infection in the eye when administered locally as drops (15,16). Since it suppresses and interferes with virus DNA synthesis, and drugs of this class have been shown to have activity against adenovirus in tissue culture (14) the topical administration of trifluorothymidine also seemed of possible clinical value. It was considered unlikely that this drug could prevent systemic infection, and it seemed unlikely that it could penetrate into the conjunctiva and remain in vascularized tissues sufficiently long to prevent conjunctivitis, but it seemed possible that it might decrease the severity of the conjunctivitis, and more important, prevent the corneal opacities which are frequently associated with adenovirus infection.

Materials and Methods. One hundred and sixty male subjects at the Florida Division of Correction, Raiford, Florida, were tested for specific neutralizing antibody to adenovirus

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type III; 28 were seronegative (17). All subjects were determined to be in good health and specifically free of liver disease, allergy, and skin eruptions.

Two drops of adenovirus type III were instilled in the lower conjunctival sac of both eyes of the 28 seronegative and two seropositive subjects. The virus which was isolated from a young male with pharyngoconjunctival fever in December, 1966, during a mild epidemic in Gainesville, Florida, was passed only once in primary human amnion cells, and stored at —70°C before use in this study.

Methisazone[3] suspension was given orally to 10 subjects in the schedule of 4 gm b.i.d. for 3 days, then 4 gm daily for 3 days beginning immediately after infection. Trifluorothymidine[4] 1% eye drops were instilled in the conjunctival sacs of both eyes of 10 other subjects in the dosage of one drop into each eye five times daily for 8 days beginning on the day of infection. Ten subjects including the two seropositive subjects acted as controls but the disease of the two seropositive subjects was considered separately. The study was carried out on a double-blind basis. Control placebo eye drops and oral suspension were used so that each subject received an oral preparation and eye drops and neither the examiners nor the subjects were informed of the code during the study.

Each subject was examined daily for 2 weeks and twice a week for 2 more weeks. Temperature, pharyngitis, cervical, and preauricular adenopathy were recorded and lid reaction, conjunctival follicular hypertrophy, hyperemia, and corneal changes were studied by slit lamp biomicroscopy and were photographed. Subjects were questioned daily for general symptoms of malaise, nausea, vomiting, abdominal pain, diarrhea, sore throat, cough, sputum, muscle ache, skin rash, and ocular symptoms of pain, foreign body sensation, discharge and lid stickiness, photophobia, tearing, and blurred vision. Investigators did not have access to the previous day's findings during their observations and questioning of subjects. In spite of the placebo, it was only possible to conduct the study on a double-blind basis because the general history of toxicity and side effects was taken by one observer, and the remainder of the examination was carried out by another observer who had no knowledge of the symptoms.

Conjunctival reaction, follicular hypertrophy, adenopathy, and pharyngitis were graded on the basis of severity and the daily scores were added and converted to a total score for the duration of the experiment so that 100 was the most severe reaction possible and 0 was no reaction at all.

Conjunctival smears and virus cultures of the pharynx and both lower conjunctival sacs were performed daily for 2 weeks and then twice weekly for 2 more weeks. Blood was drawn on a weekly basis beginning on the day prior to infection. Hemoglobin, white cell count, differential, and platelets were quantitated and bilirubin, thymol turbidity, albumin, total protein, serum glutamic pyruvate transaminase, and alkaline phosphatase were determined. The BSP determinations were done at the conclusion of the study on the group taking methisazone. Antibody studies were also performed.

Results. All subjects became clinically diseased 3 days after infection although in some, the disease consisted of only a mild preauricular adenopathy and conjunctival follicular hypertrophy. In most, the disease consisted of mild conjunctival hyperemia, and pharyngitis, moderate conjunctival follicular hypertrophy, and preauricular adenopathy. Only one subject developed a transient fever. Most subjects complained of mild photophobia, tearing, foreign body sensation during the infection and only a mild watery discharge was noted on some eyelids. By 10–14 days signs and symptoms of disease had disappeared. There was no apparent shortening of the duration of the disease in those taking methisazone or trifluorothymidine and no flare-up of disease occurred when these drugs were stopped. The two subjects with previous neutralizing antibody developed very mild clinical infection. No drug regimen completely prevented the development of conjunctivitis and pharyngitis and although subjects were not isolated, no other cases of similar disease appeared among other prisoners. In spite of

[3] Supplied by Burroughs Wellcome Company.

[4] P-L Laboratories, Milwaukee, Wisconsin.

the fact that the patient from whom the virus was isolated had mild corneal opacities, no such infiltrates were seen during this study.

The detailed and summed scores of specific daily observations are listed in Table I. Although no statistically significant differences could be determined by an analysis of variance, it was the clinical impression of all observers that the disease had been milder in some of the group taking methisazone once the code was known—three weeks after infection. Three of the methisazone group, however, developed moderate to severe clinical disease. No drug appeared to influence the

TABLE I. Scores of Daily Observations.

Subject no.	Conjunctival reaction (a)	Follicular hypertrophy (a)	Preauricular and ant. cervical adenopathy (a)	Pharyngitis (a)	Total (b)
			Methisazone group		
1	0	10	40	0	50
2	10	0	30	30	70
3	0	10	0	5	15
4	60	50	70	35	215
5	85	80	10	10	185
6	90	85	80	50	305
7	35	10	30	0	75
8	0	30	10	5	45
9	30	40	40	10	120
10	35	40	70	5	150
Av	34.5	35.5	38.0	15.0	123.0
			Trifluorothymidine group		
11	0	20	30	0	50
12	90	80	70	50	290
13	60	50	30	40	180
14	85	80	10	50	225
15	10	10	30	10	60
16	10	35	50	10	105
17	0	10	30	0	40
18	10	10	30	30	80
19	45	70	30	30	175
20	35	40	40	30	145
Av	34.5	40.5	35.0	25.0	135.0
			Control group		
21	60	70	80	35	245
22	60	70	70	50	250
23	40	40	30	10	120
24	45	40	30	30	145
25	0	10	40	10	60
26	35	50	50	30	165
27	45	50	50	0	145
28	0	40	50	40	130
Av	35.6	46.2	50.0	25.6	157.4
29(c)	5	20	30	30	85
30(c)	0	10	20	5	35
Av	2.5	15.0	25.0	17.5	60.0

[a] Each number represents the summed score of the particular sign which was studied over the 4-week period; 10-30 = mild; 40-70 = moderate; 80-100 = marked.

[b] A total of 80 or less represents minimal clinical disease.

[c] Seropositive subject.

TABLE II. Results of Conjunctival and Pharyngeal Cultures.[a]

	Methisazone group of 10	Trifluorothymidine group of 10	Control group of 8	Seropositive group of 2
No. of cultures positive for adenovirus	53/600	46/600	35/480	5/120
No. of subjects with negative adenovirus culture throughout experiment	1/10	3/10	0/8	1/2
No. of herpesvirus isolated incidentally	3	3	1	0

[a] A total of 60 cultures were obtained from each subject through the 4 weeks of the experiment. No cultures were positive until 48 hours after infection and all positive cultures were obtained within 12 days of infection.

incidence of positive cultures after infection (Table II). Positive cultures were sporadic and did not correlate with the severity of clinical disease. Herpes simplex virus was isolated on one occasion from each of 7 patients, but no clinical lesions were seen (18). Only those patients taking methisazone developed gastrointestinal disturbances and drug toxicity. Although cookies, orange juice, and coffee were given immediately after the oral medication, one subject claimed to have expectorated the medication on two occasions and three people vomited 30–60 min after taking the first dose. The methisazone caused nausea in all other subjects; and one became very nauseated, vomited frequently, and developed an elevation of his serum transaminase (SGPT); medication was withheld from this patient after the third dose for two doses. A BSP retention greater than 8% was not observed. Occasional vomiting occurred in several other subjects but not within 1 hour after drug ingestion. Aside from the nausea and vomiting and the one elevation of transaminase, no other toxicity occurred with either methisazone or trifluorothymidine.

Discussion. Trifluorothymidine was without apparent effect on course of the adenovirus infection. Neither the conjunctivitis nor the systemic disease were obviously altered by administration of this drug. Since trifluorothymidine would be expected to be hydrolyzed rapidly in vascularized tissue, and since the only effect that might really be expected in this syndrome is the prevention of corneal opacities, lack of effectiveness of trifluorothymidine in this study is not surprising. Continued studies with this drug on virus infections which produce significant corneal opacities appear worthwhile.

Methisazone is a toxic drug. In this study, very large doses of the drug were used, and all patients, even the one who expectorated the drug on two occasions probably received what would be generally considered an adequate dose (19). Only one patient developed signs of systemic toxicity, and this took the form of a transient elevation in the serum glutamic pyruvic transaminase. Although there was no indication of dangerous toxicity, virtually all patients became nauseated and many vomited at some time during the study.

Because taking methisazone is unpleasant, its use in adenovirus infection would not be justified unless very striking prophylactic or therapeutic effects were observed and smaller doses might be effective. In this study, in spite of large doses, no such striking therapeutic effect was observed. It is possible that there was a slight decrease in the severity of infection in the methisazone group, and the possibility remains that less toxic cogeners of this drug might be beneficial, but no statistically significant effect of the methisazone was apparent, and it is clear that neither the disease nor virus liberation was prevented by this drug.

The use of adenovirus type III in a volunteer study such as this provided a safe method of studying drug effect as might be predicted from previous studies (1,2). No symptoms developed in inmates of the prison not inten-

tionally infected despite the fact that the subjects were not isolated, and no serious sequellae of infection were observed. The infections all began at a known time and were rather uniform in their duration, so that a biological study on a new drug could be done with a minimum number of subjects and controls being exposed to new compounds with their uncertain toxicities. Despite previous tissue culture evidence of antiviral activity, this study did not confirm the value of trifluorothymidine or methisazone in preventing or treating adenovirus infection in man.

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Human Cytomegalovirus. Observations of Intracellular Lesion Development as Revealed by Phase Contrast, Time-Lapse Cinematography* (32863)

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Human cytomegalovirus (CMV) induces characteristic cytoplasmic and intranuclear lesions in human fetal fibroblasts. In previous reports, cultured cells infected with human cytomegalovirus were studied by cytochemistry (1), autoradiography (2), and electron microscopy (3–5). These studies provided information regarding the development of cytopathic and cytochemical effects in tissue culture related to infection by this DNA virus.

To further characterize the sequential events leading to the development of the mature nuclear and cytoplasmic lesions, human fetal fibroblasts cultured in Rose chambers were infected with CMV and observed by phase contrast microscopy. Time-lapse cinematog-

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