believe that their antibody level had dropped below that which could be detected. In contrast, the sera from 38 non-farmer's lung controls which included some healthy farmers exposed to the same dusts as the reactors, gave no precipitin lines. Likewise no precipitin lines were formed in the sera of either patients or healthy controls with trichloroacetic acid extracts of several good hays prepared in the same manner. In contrast, Pepys(6) reports that 50% of the sera from farmer's lung patients reacts with crude antigen extracts prepared by extraction of good hay and certain fungi with sodium chloride solutions. We believe that the trichloroacetic acid used for our antigen extraction and the ethanol fractionation has separated the antigens responsible for farmer's lung from other antigens commonly found in good hay and in these fungi. We conclude that farmer's lung is associated with formation of specific precipitating antibodies directed to certain antigens that can be extracted by trichloroacetic acid from moldy plant material to which the patient has been exposed.

Dr. R. Barbee has administered an aerosol of one of our farmer's lung antigen preparations to a farmer with a previous history of the disease. A few hours later, typical, acute symptoms of farmer's lung developed. The high specificity of our purified antigen preparations suggests that they may be of value in clinical diagnosis of this disease. Work is being continued on their purification and characterization.

Summary. Sera from farmer's lung patients and non-farmer's lung control individuals were reacted with trichloroacetic acid extracts of moldy hays. All the sera from patients with acute symptoms and two-thirds of the total which included recovered farmer's lung patients formed specific precipitin lines with the moldy hay extracts. No precipitin lines were obtained with sera from healthy farmers who had no history of farmer's lung or with extracts of good hay. Evidence is presented to show that farmer's lung is associated with the presence of specific antibodies in the patients' sera against antigens found in moldy hays and that these antigens are the cause of farmer's lung.

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Received April 8, 1963. P.S.E.B.M., 1963, v113.

Effect of 5-Iodo-2'-Deoxyuridine on Pseudorabies Infection in Rabbits.* (28401)

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It has been reported that local administration of 5-iodo-2'-deoxyuridine (IDU) cures or ameliorates herpes simplex keratitis in rabbits(1,2,3). This report concerns the effects of this drug on pseudorabies virus, a DNA-virus(4) which is thought to be closely related to herpes simplex virus(5), when introduced onto the cornea of the rabbit.

* This investigation was supported in part by a research grant from Nat. Inst. Health.

Materials and methods. Two concentrations of IDU were employed; a 0.1 mg%

TABLE I. Response of Rabbits Treated with IDU to Ocular Infection by Pseudorabies Virus.

Group	No. of animals	Conc of IDU (mg%)	Time in min. between treatment with IDU during (1) first 6 hr and (2) second 6 hr* (1)	(2)	Time in hr until onset of symptoms in each animal	Mean
I	4	.1	60	120	72, 52, 72, 52	62.0
II	4	.1	120	120	48, 56, 50, 56	52.5
III	8	.6	30	30	49, 53, 53, 58 57, 55, 57, 55	54.6
Control	10	—	30	30	36, 36, 36, 32, 38 36, 36, 37, 38, 33	35.8

* All animals received IDU every 2 hr for an additional period of 36 hr following the first 12-hr period.

aqueous solution,† and a saturated solution containing approximately 0.6 mg%. The latter was prepared by dissolving crystalline IDU in sterile distilled water held at 56 to 60°C and adjusted to a final pH of 7.2 to 7.5 with 1.4% $NaHCO_3$.

The Aujeszky strain of pseudorabies virus used was obtained from the American Type Culture Collection in the form of infected rabbit brain. The virus was passaged in primary rabbit kidney tissue culture cells, and the third passage of virus, having a titer of $10^{6.5}$ $TCID_{50}$/ml, was used in all experiments. Aliquots of 1.5 ml of the fluid (Eagle's MEM)(6) from the third tissue culture passage were pipetted into 5 ml screw-capped vials and stored in a dry-ice chest. As needed, a vial of the infectious fluid was thawed, and 1 drop of 10% buffered glycerol-saline added. The virus was used in this essentially undiluted form, because in preliminary experiments it was found that this concentration was necessary to induce 100% infection following direct instillation of the virus onto the sacrificed cornea.

White New Zealand rabbits weighing 1.6 to 2.0 kg were used. Following introduction of 2% lidocaine into the eye, the corneas were scarified so that 3 visible circles were produced with a 5-mm trephine set to a depth of 0.05 mm. The eyes were immediately washed with physiological saline, and after an interval of 45 minutes, 0.1 ml of the virus suspension was dropped onto the traumatized cornea. Control and IDU-treated animals were infected simultaneously in all experiments.

The animals to be treated with IDU were divided into groups, and each group was treated for 48 hours in a different manner. The IDU was administered by placing 0.1 ml in the conjunctival sac immediately prior to inoculation of the virus, 5 minutes after inoculation, and subsequently as indicated in Table I. Control animals were treated in a similar manner except that 0.85% saline was substituted for IDU.

All animals were observed at least once during the first 24 hours following inoculation of the virus, and thereafter at 30-minute intervals until symptoms of pruritus appeared. Subsequently, the animals were examined periodically until death occurred.

Results. Prior to the experiments reported here, it was found in this laboratory that when pseudorabies virus is deposited on the traumatized cornea of the rabbit's eye, there ensues an incubation period of 30 to 40 hours before a clinically apparent infection occurs. In order of appearance, early symptoms consist of ptosis, swelling and reddening of the conjunctival membrane, followed by pruritus as indicated by a tendency to scratch the eye. The pruritus increases in intensity so that the animal scratches the eye almost continuously, often mutilating the entire face. Death usually occurs 6 to 24 hours following the appearance of pruritus.

The results obtained (Table I) show that in those animals treated repeatedly with

† The IDU was supplied by Dr. Steven Sawchuk of Smith Kline and French Laboratories.

IDU there was a delay in onset of symptoms, provided the infected animals received the drug at frequent intervals. From the data obtained in these experiments, it would appear that frequency of administration was more important than concentration used since the incubation period in those animals receiving the highest concentration was not significantly greater than that for those receiving the lowest.

The data were subjected to the "t" test for significance of differences in the incubation periods noted for the controls and the treated animals. The mean incubation period for treated animals was 55.9 hours as compared with 35.8 hours for controls. The value of t obtained was 11.04. The P value was less than 0.001 for 25 degrees of freedom.

Discussion. These studies indicate that the incubation period of pseudorabies virus in rabbits, as ascertained by appearance of pruritus, is prolonged significantly following repeated administration of IDU. The delay in appearance of pruritus did not vary significantly either with the concentrations of IDU used or in the frequency of its administration, provided the infected animal received the drug at least every 2 hours.

Kaufman *et al.*(2) noted that while IDU improved the ulcerative keratitis in rabbits infected with stromal strains of herpes simplex virus, it did not reduce the mortality in these animals. In the present studies, no differences were observed in the intensity of ocular manifestations in treated and untreated animals and no animal survived the infection. In the IDU-treated animals, however, there was a prolongation of the incubation period and in most instances less mutilation of the face. The period between onset of symptoms and death in both control and test animals was variable and could not be correlated with the use of IDU.

IDU has been shown to inhibit the biosynthesis of DNA-thymine and to be incorporated into DNA as IDU-5′-phosphate in place of thymidine-5′-phosphate(7). The incorporation of IDU-5′-phosphate into viral DNA could result in a presumably "false" or noninfectious virus particle. Recently, Kaufman *et al.*(8), showed that rabbits' eyes infected with vaccinia virus could be cured with IDU, and suggested that the action of the drug might be specific through inhibition of the enzyme, DNA-polymerase, required for final assembly of viral DNA.

The data presented here do not elucidate the mechanisms by which IDU alters the pathogenesis of pseudorabies virus infections in rabbits. However, observations in this laboratory (unpublished data) that IDU does not inactivate pseudorabies virus *in vitro* suggest that IDU did not affect the intact virus particle, but rather interfered with certain reactions required for virus replication. It is possible that this interference is a temporary inhibition of the utilization of thymidine monophosphate or other nucleotides needed for synthesis of viral DNA.

Summary. When administered locally *via* the conjunctival sac, 5-iodo-2′-deoxyuridine delays the onset of symptoms of experimentally produced pseudorabies keratitis in rabbits. The incubation period was approximately 20 hours longer in treated animals than in controls.

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Received April 11, 1963. P.S.E.B.M., 1963, v113.