TABLE I.

Case No.	Date of operation	Age & relationship of donor	Age of recipient	Donor blood group	Recipient blood group	Period of ischemia (min.)
1	1-30-63	Sister (32 yr)	38	B+	A+	28
2	2-25-63	Wife (30 ")	34	A+	AB+	36
3	3-21-63	Cadaver (32 ")	21	A+	O+	105

arteriolar constriction which prevents the inflow of fluid in most renal perfusion systems (5). Finally, the benefit of mannitol in the protection of such renal grafts cannot be proven on the basis of these experiences, but there is experimental evidence that it can prevent tubular damage after deliberate experimental introduction of blood pigments and by-products into the blood stream(6).

Summary. Three cases of clinical renal transplantation have been performed, using donor organs of different blood type than that in the recipient patient. Graft function occurred in all 3 cases, being most retarded in the kidney subjected to the longest ischemia. The kidneys provided by living donors have essentially normal function 9 and 5 weeks after transplantation.

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Antigens in Moldy Hay as the Cause of Farmer's Lung.* (28400)

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Farmer's lung is a potentially serious and crippling occupational disease that is associated with the inhalation of dust from a variety of moldy plant materials, most frequently forage. The symptoms have been described as shortness of breath, cough, fever, chills, weight loss, and hemoptysis associated with an acute granulomatous interstitial pneumonitis(1). The physiopathological features of farmer's lung have been recently described by Rankin *et al.*(2). Although farmer's lung was first described by Campbell(3) over 30 years ago, the exact cause of this disease has not been clearly established. It has been suggested that it results from mechanical irritation or to fungal infection. Symptoms appear several hours after exposure to the dust and increase in severity upon repeated exposures. Dickie and Rankin(1) suggested that farmer's lung may arise from a hypersensitivity to molds or to the products of molds occurring in a wide variety of organic material.

In late 1960, we started experiments designed to detect specific precipitating antibodies against antigens from moldy forage in sera of patients with farmer's lung. Precipitins against antigens extracted with trichloroacetic acid from moldy hays from patients' farms were demonstrated in the sera of all farmer's lung patients who were acute-

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ly ill and in most of the asymptomatic farmers who had previously suffered from this disorder. A preliminary report of these immunological findings has been given by Stahmann and Kobayashi(4). Independently and about the same time, Pepys and associates (5) also demonstrated precipitins against saline extracts of hay and of molds in the sera of patients with farmer's lung, pulmonary infiltration associated with hypersensitivity to aspergillus fumigatus, and sarcoidosis. The sera of the farmer's lung patients examined by Pepys *et al.*(6) reacted with extracts of moldy hay and half of them also reacted to extracts of good hay or to antigens extracted from a variety of fungi. Their moldy hay extracts also reacted with sera from some normal subjects. Our studies have been directed toward the detection and extraction of antigens specifically related to farmer's lung which would not react with sera from normal subjects. This paper describes results of precipitin tests with trichloroacetic acid extracts from moldy forages obtained from patients' farms that gave strong precipitin reactions with sera from all acutely ill patients yet gave no reactions with control sera from healthy farmers. Extraction of the antigens and immunochemical studies were carried out in the Biochemistry Dept.

Materials and methods. Sera were collected from patients in whom a firm diagnosis had been established by appropriate clinical, physiologic or pathologic studies. Some sera were collected during the acute phase of the disease, and the remainder during a relatively asymptomatic interval following a typical attack 1-18 years previously. Most of these patients have suffered from recurrent episodes of respiratory illness and have been extensively studied at intervals over a period of years. Control sera were obtained from both farmers and non-farmers and patients with other pulmonary disorders. Moldy hay and silage samples responsible for illness in a farmer were obtained from Wisconsin farms; some moldy hay samples, and samples of good hay and silage were obtained from the University farm.

In the initial experiments, moldy hay samples were extracted with a variety of solvents and the extracts tested for their ability to form precipitates with sera from farmer's lung patients using the double diffusion technique of Ouchterlony(7). The solvents studied for their ability to extract specific antigens included 0.9% sodium chloride containing 0.5% phenol, 0.1 N acetic acid, pH 7.4 phosphate buffer, 0.1 N sodium hydroxide, 50% phenol, and 0.5 M tricholoroacetic acid. Although several of the solvents extracted antigens which gave precipitin reactions with farmer's lung sera, tricholoracetic acid was generally used because it gave strong precipitin reactions and was the most selective solvent. The trichloroacetic acid in the extract was removed by dialysis and the antigens precipitated with 90% ethanol and dried with acetone. The resulting antigen preparations were tested against various sera by double diffusion in agar gels(7), and by immunoelectrophoresis in agar(8).

Some typical results obtained in double diffusion experiments are illustrated in Fig. 1 and 2. In Fig. 1, the center well contained the antigen preparation from a moldy hay sample obtained from the farm of patient H. The top outer well contained sera from a control, normal individual; the other 5 outer wells contained sera from 5 typical patients. This antigen preparation formed a precipitin line with the sera of all 5 patients, but no line was produced with the normal sera.

In Fig. 2, the large center well contains serum from a farmer's lung patient (W). The 6 outer wells contain the moldy hay antigen preparations. Wells No. 1-3 represent those prepared from moldy hay samples obtained from Wisconsin farms. Well No. 4 contains the antigens from a sample sent to us by Dr. P. H. Gregory, Rothamsted Experimental Station, Harpenden, England,‡ that was produced by inoculating cold sterilized, good quality hay with a spore suspension obtained from hay known to cause farmer's lung in England. Well No. 5 contained antigens from

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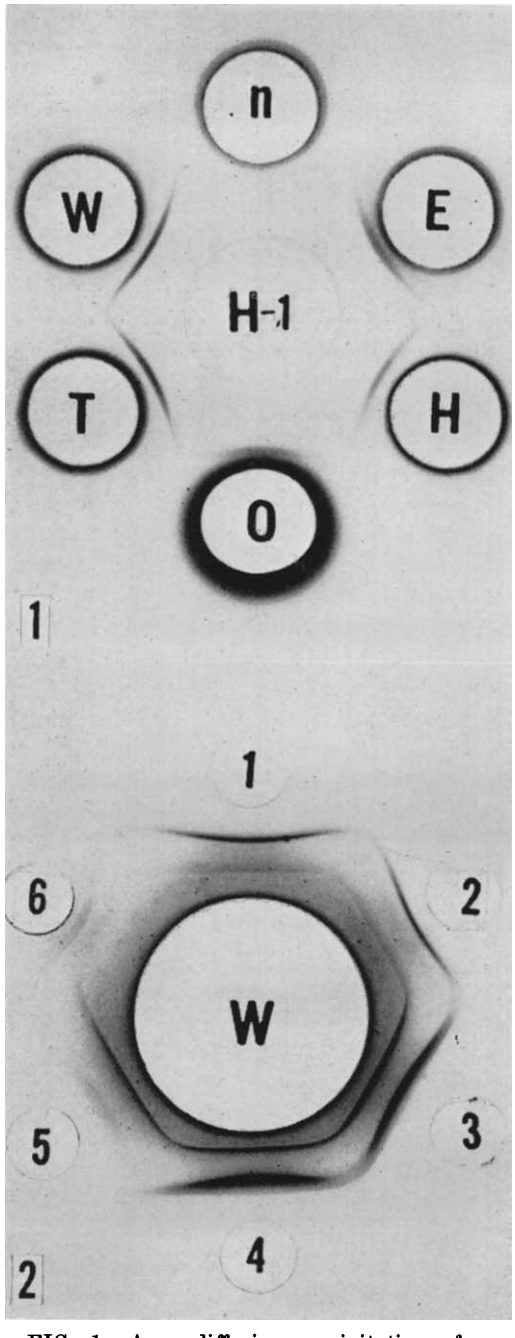

FIG. 1. Agar diffusion precipitation of sera with farmer's lung antigens. Center well contained farmer's lung antigen (15 mg/ml) extracted from moldy hay (H-1). The outer well, n, contained normal serum; wells E through W contained different farmer's lung sera.

FIG. 2. Agar diffusion precipitation of antigens with farmer's lung serum. The center well (W) contained farmer's lung serum; each outer well contained 15 mg/ml of an antigen preparation. Well No. 1 contained antigen preparation from moldy hay No. MH-1; No. 2, UH-1; No. 3, H-1; No. 4, H-65; No. 5, M; and No. 6, MH-44.

moldy hays prepared by Dr. Gregory by inoculation with a mixture of known fungi. Well No. 6 contained an antigen extract obtained from Dr. J. Pepys, Inst. of Diseases of the Chest, London,‡ that was prepared from English hay known to cause farmer's lung. Each of the preparations formed one or more precipitin lines. Some of these lines seemed to merge at their junction suggesting common antigens in the different moldy hay samples.

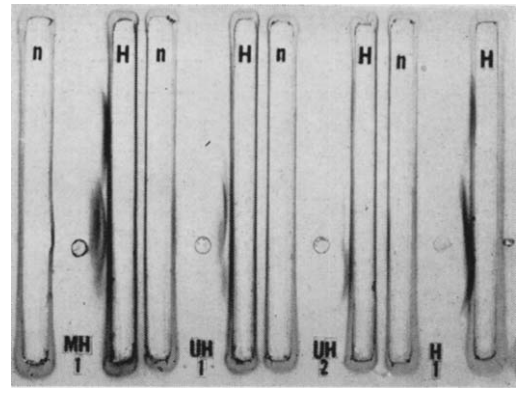

FIG. 3. Agar immunoelectrophoresis of sera with farmer's lung antigens. The small wells labeled MH-1, UH-1, UH-2, and H-1 contained 15 mg/ml antigen; troughs labeled N contained normal serum; those labeled H were filled with farmer's lung serum.

Fig. 3 shows typical results obtained on immunoelectrophoresis of the antigens prepared from 4 different moldy hay samples. The antigens were reacted with serum from patient H and normal sera labeled N. No precipitation lines were formed with the normal control sera. The farmer's lung serum gave precipitin lines with all 4 moldy hay extracts. There were differences in the number, position, and intensity of the precipitin line from the 4 different hays. That from moldy hay labeled MH-1 showed 3 strong precipitin lines. The other 3 hay samples gave fewer and weaker lines although they were in the same relative positions.

We have examined sera from 36 patients in whom a firm clinical diagnosis of farmer's lung had been established by extensive clini-

TABLE I. Correlation Between Clinical Diagnosis and Immuno-chemical Precipitation.

Clinical diagnosis	No.	Immuno-chemical precipitation* ++	+	—	Reactors %
1. Patients					
a) symptomatic	7	7	0	0	100
b) asymptomatic	29	6	11	12	59
c) Total	36	13	11	12	67
2. Controls	38†	0	0	0	0

* Against a mixture of antigens from 4 moldy hays.

† Farmers and patients with other pulmonary diseases.

cal, roentgenographic, physiologic, and in some instances, pathologic studies. Seven of these sera were collected from acutely ill patients and the remainder from asymptomatic farmers who had suffered from this disorder during the past 1-18 years. These 36 sera and 38 controls collected from farmers exposed to the same dusts as well as patients with sarcoidosis, fungus infections, and other unrelated pulmonary diseases were reacted with a mixture of antigens prepared from 4 moldy hays. The results of these experiments are shown in Table I.

Discussion. We have examined some moldy hay samples thought to have been responsible for the disease in a farmer and others from farms where this disease has not occurred. In addition, we have tested samples of good hay. A few moldy hay samples from England including those that were produced by Dr. P. H. Gregory by inoculation of sterilized hay were also studied. Precipitin lines were produced by the antigens extracted from most, but not all, of the moldy hay samples when reacted with one or more of the sera from farmer's lung patients. No precipitin lines were produced by similar trichloroacetic acid extracts from any of the good hays which were not moldy. Sera from healthy controls and patients with other pulmonary disorders gave no precipitin lines with the moldy hay antigens. The results (Fig. 3), suggest that there may be a small number, possibly 1, 2 or 3, antigens associated with farmer's lung. Fig. 1 shows that the sera of 5 patients form a continuous precipitation line suggesting that they all have antibodies that react with the same antigen. Fig. 2 shows that 2 antigens are common to 3 moldy hay samples, MH-1, UH-1, H-1 collected from farms of patients in Wisconsin for 2 continuous precipitation lines are formed. The same 2 antigens seem to be present in a moldy hay sample (H-65) sent to us by Dr. P. H. Gregory who produced it by inoculating sterilized, good quality hay with a spore suspension from moldy hay known to cause farmer's lung in England or with a mixture of known fungi (M). These results suggest that the farmer's lung antigens are produced by an interaction of the fungi and the plant material.

By immunoelectrophoresis of patients' sera and subsequent precipitation with the moldy hay extracts, we showed that only the γ-globulin fraction of the sera formed precipitates with the moldy hay antigen. The amount of the farmer's lung antigens in the extracts from different moldy hays varied widely. This was estimated by reacting serial dilutions of the antigen extracts with a potent sera from a farmer's lung patient. Some precipitin lines disappeared after a 2-fold dilution of the extract; others persisted even after the antigen extract was diluted 128 times. From such results we conclude that the relative precipitating activity or amount of the antigen in moldy hay samples may differ by many fold.

In a similar way we determined the antibody level in several patients' sera by estimating their dilution endpoint with antigen preparations. The dilution endpoints of 4 sera varied from 32 to 128 when tested with one antigen preparation. It was lower with some other sera. From such results, it is clear that the antibody level in patients' sera may also vary greatly.

The data of Table I show that sera from all who showed acute symptoms at the time blood was taken gave strong precipitin lines. Of 36 patients in whom a firm diagnosis had been established, 13 gave strong precipitin lines, 11 gave weak precipitin lines and 12 did not form visible precipitin lines. Among those 12 were patients who had not shown symptoms for the past several years and some who had left the farm completely. We

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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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.

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