monary edema. None of these rats developed the usual acute edema, and in 10 cases the lungs had normal weight. This result is interpreted as a proof of the central nervous mediation of the acute pulmonary edema produced by administration of epinephrine.

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Passive Transfer with Circulating Leukocytes of Delayed Hypersensitivity to Cat Scratch Antigen.* (22723)

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The passive transfer of delayed type skin hypersensitivity in animals and in man has been demonstrated towards simple chemical compounds(1), to tuberculin(2), to whole streptococcal cells, to SK-SD, and to streptococcal M substance(3,4). Such transfer has been possible with the leukocytes from peritoneal exudates, from lymph nodes, from the spleen, and from the thoracic duct, and from certain fractions of disrupted leukocytes(5,6). Hitherto the passive transfer of skin hypersensitivity to virus diseases has not been reported. It is the purpose of this paper to report the successful passive transfer of the delayed type skin hypersensitivity to cat scratch disease by means of circulating leukocytes. Cat scratch disease is a benign, subacute, regional lymphadenitis that is characterized by reticuloendothelial hyperplasia with epithelioid cell granulomas having central necrosis. Patients with cat scratch disease develop a characteristic skin reaction to an antigen prepared from pus aspirated from other cases of cat scratch disease. In its temporal relationships and its morphological features the reaction to cat scratch antigen has features similar to the tuberculin reaction and is characteristic of delayed allergy in man. No causative agent has been isolated from the suppurating lymph nodes from patients with cat scratch disease despite cultures on all commonly used media, upon inoculation into laboratory animals including rabbits, cats, monkeys, guinea pigs, mice, rats, and chick embryos, or on inoculation of tissue culture of HeLa cells, kidney cells, liver cells, or corneal cells.§ No microorganisms are to be seen in sections of the involved lymph nodes although cytoplasmic basophilic inclusion bodies have been described in the cells of such nodes(7). Further evidence in support of a viral etiology for cat scratch disease is that patients with this disease have a distinctly higher incidence of low complement fixation titers against the lymphogranuloma-psittacosis group viruses than does the population at large(8).

Materials and methods. Obtaining white blood cells. The antecubital area is cleansed with tincture of iodine, alcohol and green soap. The skin is infiltrated with 1% procaine hydrochloride. The antecubital vein is punctured with a 15 gauge needle and the blood is withdrawn through silicone-coated tubing into a silicone coated vacuum bottle containing ACD as anticoagulant. (For volumes of blood less than 400 ml heparin, 5 mg/100 ml blood, is used as the anticoagulant). Twenty-five ml of 4.7% dextran is

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TABLE I.

Donor sensitive to cat scratch disease antigen			Recipient not sensitive to cat scratch disease antigen			No. of viable cells transferred (millions)	Mode of inoculation	Height of reactivity	Duration of reactivity, wk
	Sex	Age		Sex	Age				
C. D.	♀	11	M. M.	♀	3	260	Intramusc. + subcut.	2+	3
P. D.	♀	8	S. M.	♂	5	400	Intramusc. + subcut.	3+	>4
M. D.	♀	46	G. A.	♂	8	1000	Intramusc.	+	2

added for each 100 ml of blood, and the mixture inverted twice. (If heparin is used, normal saline is added in addition to the dextran, 29.5 ml/100 ml of blood). The mixed blood is allowed to stand at room temperature for 50 minutes, while red cells settle at a rate far in excess of the rate for the white cells. The plasma containing the white blood cells is removed with a plasma separating needle and a silicone-coated vacuum flask. The plasma is placed in silicone-coated centrifuge tubes and centrifuged at 1,000 rpm for 20 minutes. The supernatant plasma is poured off and the leukocytes together with about an equal number of admixed red cells are suspended in normal saline. The suspension is taken up into a silicone-coated syringe and injected intramuscularly, or partly intramuscularly and partly subcutaneously. *Handling the white blood cells.* An aliquot of the white cell suspension is used to determine the total and differential counts, and the percentage of viable cells is estimated by vital staining with trypan blue(9). Approximately 200,000,000 white blood cells are obtained/each 100 ml of blood processed, and between 80 and 99% are viable. The cell donors were patients convalescent from cat scratch disease who demonstrated a strongly positive skin test to cat scratch disease antigen. The recipients were children who had negative skin tests for cat scratch disease. Matching for compatibility of red cell groups was carried out because of the admixture of erythrocytes with the leukocytes. The cat scratch antigen was prepared from sterile pus aspirated from 2 carefully characterized cases of cat scratch disease. The pus was diluted 1:5 with pyrogen free normal saline and heated at 56 degrees C for one hour on 2 consecutive days. The resultant antigen in 0.1 ml quantities gave reactions characterized by induration and erythema beginning at 12 hours and lasting for 72 hours to 5-6 days after intradermal injection in subjects with typical cat scratch disease. Reactions on controls were completely negative. In the experimental series, skin tests were applied two days after the transfer of cells, and at weekly intervals thereafter. These tests were read for induration and erythema at 48 hours, as follows: +, 5 to 10 mm; 2+, 10 to 15 mm; 3+, 15 to 20 mm. Ancillary evidence of hypersensitivity was shown by the occurrence of a brownish discoloration after the erythema had faded. This discoloration, which persisted for many weeks in patients with cat scratch disease, lasted as long as the passively transferred reactivity could be demonstrated.

Results. The results summarized in Table I demonstrate the successful passive transfer of cat scratch disease skin hypersensitivity by means of circulating leukocytes in three consecutive cases.

An attempt to transfer the skin reactivity to cat scratch disease by means of plasma from a convalescent patient with a strongly positive skin reaction (4+) was unsuccessful. Four hundred cc of blood were removed from patient M.D. into a standard blood collecting flask with ACD as anticoagulant. After settling of cells the plasma was removed and filtered through a Seitz filter. One hundred and fifty cc of this plasma were given intravenously to recipient M.M. This patient failed to develop positive skin reactivity to cat scratch disease antigen.

Summary. Delayed type hypersensitivity to cat scratch disease can be passively transferred by blood leukocytes, but not by plasma from hypersensitive donors convalescent from

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Effects of Azetazoleamide (Diamox) Upon Blood Sugar of Normal and Diabetic Patients. (22724)

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Moseley *et al.*(1) observed insulin shock in a diabetic patient receiving azetazoleamide as a diuretic and subsequently made studies on normal subjects to evaluate the effect of this drug on glucose tolerance. No significant change in the intravenous glucose tolerance curve was noted when studied with and without the administration of azetazoleamide. They concluded from the oral glucose tolerance curve that azetazoleamide may interfere with the absorption of glucose from the gastrointestinal tract.

Since Moseley did not study diabetic patients and limited his study to an acute problem, our studies were designed to determine whether azetazoleamide influences the fasting blood sugar of normal and diabetic patients after acute and chronic administration and whether the oral glucose tolerance curves can be affected.

Acute study. Acute experiments were carried out in 20 normal and 20 diabetic subjects. Half of each group were given either 500 mg of azetazoleamide* orally and fasting blood sugars determined (method of Folin-Wu)(2) 3 hours later or followed without drug administration for a similar period. One week later, to eliminate biologic variation, the subjects were re-studied but the procedure reversed. The results of this experiment are summarized in Table I. Azetazoleamide did not alter the fasting blood sugar 3 hours after its administration in the normal and the diabetic group of patients when studied as an acute experiment. The blood sugar taken at 3 hour intervals was practically the same with and without the drug.

Chronic study. Chronic experiments were carried out in 6 normal and 6 diabetic patients. Azetazoleamide was given in doses of 500 mg daily for a 3 week period to 3 normal and 3 diabetic patients. Fasting blood sugars were determined (method of Somogyi)(3) at weekly intervals. As a control, a similar group was followed without drug administration. In Table II average values of blood sugars are recorded. With chronic administration of 500 mg of azetazoleamide there was no significant change in the fasting blood sugar in either the control patients or those receiving the drug for a period of 3 weeks.

Oral glucose tolerance. The final experiment was related to the effect of azetazoleamide on the glucose tolerance curve. Three normal and 3 diabetic patients received the standard oral glucose tolerance test utilizing 100 g of glucose. The procedure was repeated at a later date with 500 mg of azetazoleamide being given 3 hours prior to the

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