hearts and samples of adipose tissue, skeletal muscle and plasma were taken for determination of total C^{14} and its distribution among glycerides, phospholipids, cholesterol esters and free fatty acids. At all intervals the ratio (C^{14} in phospholipid/C^{14} in glycerides) was greater after injection of C^{14}-labeled stearic acid than after that of labeled palmitic acid. This indicates that preferential incorporation of stearic acid into phospholipids is not limited to incorporation during absorption from the intestines into lymph.

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Heterogeneity of Antinuclear Factors in Lupus Erythematosus and Rheumatoid Arthritis.* (29976)

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Antinuclear factors (ANF) of γ_1, γ_1A, and γ_1M immunoglobulin classes (IgG, IgA, and IgM)‖ have been found in the sera of children and adults with lupus erythematosus (LE) and rheumatoid arthritis (RA)(1). These 3 immunoglobulin classes were distinguished on the basis of their antigenically differing heavy (H) chains. All 3 immunoglobulin classes have similar light (L) chains in common. Antigenically, however, L chains and the individual immunoglobulin molecules which carry them may be divided into 2 distinct types, L I and L II(2) (κ and λ)‖. The present study demonstrates that immunoglobulins with an ANF activity include molecules of both L chain types, as detected by a 3-layer indirect immunofluorescence test.

Materials and Methods. The 3-layer im-

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‖ Nomenclature for Human Immunoglobulins, Bull. Wld. Health Org., 1964, 30, 447.

TABLE I. Immunoglobulin Classes and L Chain Types of Antinuclear Factors in 26 Human Sera.

Diagnosis	Serum	H chain detected			L chain detected	
		$\gamma 2$	$\gamma 1A$	$\gamma 1M$	I	II
LE	A	+	—	—	+	+
	D_1	+	+	+	+	+
	E	+	+	+	+	+
	S_1	+	+	+	+	+
	S_2	+	+	+	+	+
	S_3	+	+	+	+	+
	V	+	+	+	+	+
	It	+	+	+	+	+
Adult RA	N	+	—	+	+(+)	—(+)
	P_2	—	—	+	±(+)	±(±)
	S_8	+	—	—	+	+
	F	—	—	+	+	+
	M_2	+	—	+	+	+
	H_2	+	—	+	+	+
	H	+	+	+	+	+
	B	+	+	+	+	+
	S_7	+	+	+	+	+
	H_1	+	+	+	+	+
Juvenile RA	RM	+	—	+	—(+)	+(+)
	JA	+	—	—	—	+
	LK	+	—	—	+	+
	LR	—	—	+	—	—
	AS	—	—	+	+	+
Chronic hepatitis	Si	+	+	+	+	+
	He	+	+	+	+	+
Psoriatic arthritis	Ta	+	—	+	+	+

+ = ANF positive.
± = ANF equivocally positive.
— = ANF negative.
( ) = result given with patient's serum after 2-4 fold concentration.

munofluorescence test, as previously described (1), uses human white blood cells as a source of nuclei. Peripheral blood smears were fixed in 95% alcohol. The patient's serum or a fraction thereof was then layered onto one or more slides as the first layer. The slides were then washed, and a rabbit antiserum specific for one of the human immunoglobulin (H chain) classes or (L chain) types was then added. After a second wash, a goat anti-rabbit globulin conjugated with fluorescein isothiocyanate was used as the third layer. The appropriate controls were run in each test to demonstrate that the goat anti-rabbit globulin did not cross-react with human serum protein. The fluorescence microscope used was as previously described(1).

The methods of preparation of the rabbit antisera to the immunoglobulin classes have been previously described(3). Antisera specific for type I or type II Bence Jones proteins were employed to detect type I or type II L chains(4). Rabbits were immunized with Bence Jones (BJ) proteins in complete Freund's adjuvant. The rabbit antisera were serially absorbed with cross-reacting human antigens, *i.e.*, with the opposite type of BJ protein and with serum myeloma proteins of the opposite L chain type, until they gave only single lines in Ouchterlony double diffusion with homologous antigens. The specificities of these antisera were confirmed in the quantitative complement fixation test of Wasserman and Levine(5), by passive cutaneous anaphylaxis in guinea pig skin(6) and by specific blocking of the immunofluorescent ANF test with appropriate antigens.

Results. Twenty-six patients' sera were investigated for the L chain types exhibited in their ANF, as detected by the use of the specific anti-BJ sera. These patients' sera, previously shown to contain ANF of one or more immunoglobulin classes(1), (Table I) were from 8 adults with LE, 10 adults with RA, 5 children with RA, 2 adults with chronic active hepatitis, and one adult with psoriatic arthritis. All 8 LE sera tested were shown to contain ANF having type I and type II L chains when the sera were tested undiluted. This was also true of 8 of 10 sera from adult RA patients, 2 of 5 sera from juvenile RA patients, and 3 sera from patients with other diagnoses.

Of the 5 sera which at first did not appear to have both type I and type II ANF, one (serum N) appeared to have only type I even though ANF activity was detected in both γ_2 and γ_1M immunoglobulin classes. Serum P_2 gave equivocal nuclear fluorescence with both type I and type II antisera. In sera RM and JA only type II ANF was detected. Sera from patients P_2, N, and RM, which were the only sera of the 5 in sufficient quantity, were concentrated 2 to 4 times by pervaporation. After concentration, both types I and II L chains were detected in sera N and RM. Serum P_2 gave only equivocal fluorescence with the anti-type BJ II serum. Serum LR with γ_1M ANF in low titer failed to give nuclear fluorescence with either anti-L chain sera. Thus, of 26 sera tested, only sera P_2, JA, and LR could have had ANF of only

one L chain type. Unfortunately the short supply of these did not allow further concentration experiments.

Sera A, S_8, and LK contained ANF only in the γ_2 immunoglobulin class, but they were shown to have ANF of both L chain types. Sera F and AS contained γ_1M ANF alone, but again both type I and type II ANF were present. Four LE sera exhibiting ANF in more than one immunoglobulin class were fractionated by diethylamino ethyl cellulose chromatography(7) and in all 4 cases eluates shown to contain γ_2 or γ_1M ANF alone also contained both type I and type II L chains.

Discussion. The anti-BJ sera used in this study have been shown to be capable of detecting type I and type II L chains if they are present in sufficient quantity on the white cell nucleus, or on the red cell membrane(3). However, anti-BJ sera were less effective when used as Coombs(4) or immunofluorescent reagents than were anti-H chain sera.

Twenty-three of 26 sera containing one or more immunoglobulin classes of ANF were shown to have both type I and type II L chains in the ANF. Sera, or chromatographic fractions of sera, which exhibited only one immunoglobulin class of ANF contained ANF with both type I and type II L chains. Thus, heterogeneity in respect to L chains has now been demonstrated for antinuclear factors, in addition to heterogeneity in respect to immunoglobulin class(1). The L chain heterogeneity is like that already known for human Rh antibodies(4,8,9), rheumatoid factors(8), thyroglobulin autoantibodies(9,10), some red cell autoantibodies(4), and antibodies to dextran and teichoic acid(9).

One explanation for the heterogeneity of ANF may be that these antibodies are produced by a rather unrestricted, or heterogeneous, population of plasma cells. Given plasma cells have been shown almost always to contain only one H or L chain type(11,12). The production of ANF thus seems likely to involve many clones of plasma cells, such as characterize normal antibody formation. The hypothesis, therefore, that ANF autoantibodies arise by somatic mutation(13) seems difficult to support. This is especially so, when one considers also the numerous other autoantibodies that have been found in sera containing ANF. Such a large array of autoantibodies may be better imagined to reflect abnormal release of tissue antigens than the function of abnormal clones of antibody producing cells. On the other hand, some autoantibodies, such as "rheumatoid factor" reported in the serum of a patient with lymphoma(8), cold agglutinins(8), and the red cell autoantibodies in a significant number of patients with acquired hemolytic disease(4) have been found to be strikingly homogeneous with respect to L chain type. This suggests that these particular autoantibodies may be produced by sharply restricted populations of plasma cells, somewhat analogous to the production of antigenically homogeneous proteins by abnormal cells in the paraproteinemias. The mechanisms responsible for homogeneity are at present matters of speculation only, but it is possible, as suggested by Allen, Kabat, and Kunkel(14), that minimal stimulation of the antibody forming tissue by certain unusual antigens, such as those having sharply limited antigenic configurations, may stimulate only a restricted population of plasma cells. Antigens with greater configurational differences, it is supposed, would induce antibody formation in a wider variety of cells. It is of note, in this respect, that ANF immunofluorescence has been shown to have specificity for several different nuclear constituents(15), and that this alone may account for the heterogeneity of ANF described herein.

Summary. Twenty-six sera from adults and children with lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, or chronic active hepatitis were tested in a 3-layer immunofluorescence test on human white cell nuclei. Rabbit antisera against human type I and type II Bence Jones protein were used to determine whether the antinuclear factors contained type I or type II L chains. Twenty-four of the sera were shown to contain ANF with both types of L chains. In the remaining 2 cases quantitative considerations limited the study. Both type I and type II ANF were also found in isolated fractions of γ_2 and γ_1M globulins in 4 lupus sera.

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Determination of Antialdosterone Activity in Normal Dogs. (29977)

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Compounds having aldosterone-antagonistic activity are generally screened and evaluated in the laboratory by their ability to reverse the depressed urinary Na/K ratio of adrenalectomized rats given desoxycorticosterone acetate (DCA)(1). This report describes a method for detecting and evaluating aldosterone antagonists in a second laboratory species. The test is based upon the ability of an antagonist to reverse the depressed urinary Na/K ratio seen after administration of 9 α-fluorohydrocortisone (9FHC), a potent mineralocorticoid, to intact unanesthetized dogs.

Materials and methods. A colony of 15 trained, unanesthetized female hound dogs maintained at constant weight (18-25 kg) on Purina Lab Chow were used in all these experiments. The dogs were fed at 4:00 to 4:15 p.m. every afternoon. Water was allowed *ad libitum* except during the experiment. The dogs were housed in individual cages in a room having automatic constant control over temperature, humidity, and artificial lighting. Generally, 10 to 12 dogs were used on one day for each dose of compound. On the control and test days the animals were catheterized with a urinary retention catheter. The end of the catheter was clamped with a pinchcock and fixed around the dog's body with string, allowing subsequent urine collections without recatheterization. The bladder was emptied and, on test days, the animals were given an intramuscular injection of 9FHC in 0.25 ml of corn oil. The compound to be tested was then immediately given orally by means of a stomach tube in a small volume of water. Urine was collected and the total volume measured every 2 hours over a 6-hour period. Sodium and potassium were determined with a Baird Atomic Model KY-1 flame photometer adapted for automatic flow type analysis. Chloride was determined with a Technicon autoanalyzer.

Despite controlled experimental conditions, the normal Na/K ratio of the colony showed considerable variation when measured periodically over a period of one year. The least variation occurred when the Na/K ratio was determined on 2 successive days (Table I, control row). Therefore, for all subsequent tests a control 6-hour urine collection in which nothing was given to the animals was always obtained the preceding day. This control Na/K ratio was then used for statistical comparison with test day results.