segment depression. In isolated T-wave inversion the method is not applicable.

Discussion. This follow-up of 226 cases of ST junction depression provides additional evidence that ST junction depression is a normal response to exercise. Even the prolonged and "near ischemic" types ascribed by some investigators to mild coronary insufficiency had a low death rate. In contrast, all grades of ischemic ST segment depression had a high coronary death rate. This type of electrocardiographic response to exercise evidently is due to coronary insufficiency caused by coronary atherosclerosis. The increase in coronary death rate that accompanies the increase in ST segment depression suggests that degree of ischemic depression is directly related to severity of coronary insufficiency induced by the test. It is of interest that the 8 coronary deaths in the Grade 2 and 3 cases showed sagging ST segments and diphasic or inverted T-waves. This type of reaction probably denotes a more severe degree of coronary insufficiency than does the horizontal type. Further analysis according to type and degree will be made when enough material is available. While QS, QT ratios differ for the extremes—negative reactors and those with ischemic ST segment depression—this technic does not offer any special advantage over inspection of the tracings.

Summary. Ischemic ST segment depression after exercise is due usually to coronary atherosclerosis and insufficiency. Our data suggest that type and degree of ischemic ST segment depression after exercise reflects the severity of the coronary disease. The results of this investigation indicate also that ST junction depression is a normal response to exercise.

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Immunocytochemical Demonstration of Intranuclear Localization of 18S Gamma Macroglobulin in Macroglobulinemia of Waldenström (25555)

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We recently reported the presence of intranuclear material within lymphoid plasma cells of 3 patients with macroglobulinemia of Waldenström(1). This material was histochemically identified as a protein with high carbohydrate content. The carbohydrate component was shown not to consist of glycogen since it was periodic acid-Schiff(PAS) positive after digestion with diastase. The isolated serum 18S gamma macroglobulin from each of the 3 patients also had a high carbohydrate content and was strongly PAS positive following electrophoresis on filter paper strips.

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The present study, utilizing the fluorescent antibody technic of Coons and Kaplan(2), supports the probable identity of protein material within the nucleus and cytoplasm of lymphoid plasma cells and the circulating serum 18S gamma macroglobulin from a patient with macroglobulinemia of Waldenström.

Methods. Antisera preparation: Purified gamma macroglobulin from serum of patient J.W. was obtained by repeated precipitations of the protein from solution by dialysis against low ionic strength pH 8.0 buffers. The final product was homogeneous by analytic electrophoresis and upon ultracentrifugation consisted of 18S macroglobulin (with small

amounts of 23S and 27S components). Whole normal human gamma globulin, prepared by electrophoretic procedures contained 90% 6.6S gamma globulin and 10% 18S gamma macroglobulin(3). Antisera to purified J.W. macroglobulin and to whole normal human gamma globulin were produced utilizing technics described elsewhere(3). Rabbit anti-macroglobulin serum was absorbed with commercially prepared gamma globulin (Cohn Fraction II) to remove antibodies to 6.6S gamma globulins. This absorbed anti-macroglobulin serum produced single precipitin bands in Ouchterlony gel diffusion plates(4) when tested against purified J.W. macroglobulin and against whole normal gamma globulins. These bands showed a reaction of identity in the gel diffusion plate demonstrating specificity of the absorbed rabbit anti-macroglobulin for the J.W. macroglobulin and for at least some of the 18S gamma macroglobulin present in whole normal gamma globulin. Rabbit anti-whole normal gamma globulin serum contained antibodies against both 6.6S and 18S gamma globulins. This antiserum was absorbed with purified J.W. macroglobulin to removed antibodies which might have reacted with J.W. macroglobulin. The antibodies in the 2 absorbed antisera were precipitated and concentrated by ⅓ saturation with $(NH_4)_2SO_4$ redissolved and dialyzed against saline. We are extremely grateful to Dr. Arthur Silverstein, Chief, Immunochemical Section, Armed Forces Inst. of Pathology, for performing the fluorescein labeling of the concentrated antisera. The labeled antisera were absorbed with acetone dried human liver powder. *Bone Marrow Smears*: At the time of our original report, J.W. did not have demonstrable intranuclear PAS positive material in cells aspirated from bone marrow but this has subsequently been observed in repeated marrow aspirates. Aspirated marrow particles were smeared on coverslips, air dried and stored at 4°C until stained. The coverslips were placed in 4°C acetone for 10 minutes, rapidly air dried and covered with absorbed fluorescein labeled antisera for 45-60 minutes in a moist chamber at room temperature. They were then agitated gently during two 10 minute rinses in pH 7.4 phosphate buffered normal saline, mounted on slides with 25% glycerol in buffered saline and observed with Leitz ultraviolet microscope using wavelength of 390-440 mμ. Several cells with intranuclear fluorescence were photographed and the microscopic fields were marked. The coverslips were removed from the slide by immersion in buffered saline, air dried, fixed for 3-5 minutes in formalin vapor, washed in running water and stained by a PAS-Hematoxylin technic. The marked fields were then examined with standard illumination. Controls for specificity of fluorescence consisted of bone marrow smears from J.W. incubated with the absorbed fluorescein labeled anti-normal gamma globulin and smears incubated with pooled rabbit sera containing unlabeled anti-macroglobulin for 1 hour prior to incubation with the fluorescein labeled anti-macroglobulin. Smears of aspirated bone marrow particles from patients with multiple myeloma and carcinoma served as non-macroglobulinemic controls.

Results. Specific, brilliant green fluorescence was present within the otherwise non-fluorescing nucleus of several cells in smears incubated with the fluorescein labeled anti-macroglobulin preparation (Fig. 1). Diffuse specific fluorescence was also present in cytoplasm of most cells with intranuclear fluorescence and in cytoplasm of a few cells with an eccentric, non-fluorescent nucleus. Preincubation with unlabeled anti-macroglobulin serum diminished intensity of the specific fluorescence. The fluorescent nuclear area was usually demonstrated to be PAS positive.

Some cells with the intranuclear area of fluorescence were noted to have a zone of perinuclear fluorescence which was not sharply circumscribed (Fig. 2). In PAS stained preparation, these cells were distorted and consisted of an apparently intact nucleus with a surrounding clear zone, within which there were clumps of PAS positive material but no recognizable cytoplasmic structures. Most cells of this type contained intranuclear PAS positive material but in a few the area previously occupied by specific fluorescence was colorless. It is assumed that the macroglobulin was in this intranuclear area at the time of the reaction between it and the labeled

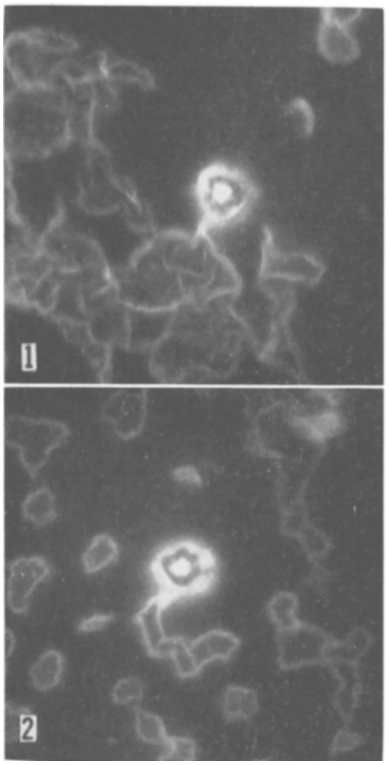

FIG. 1. Fluorescence photomicrograph of bone marrow smear incubated with fluorescein labeled anti-18S macroglobulin. Compact fluorescence in nuclear area and surrounding diffuse, but rather well circumscribed cytoplasmic fluorescence. Note erythrocytes outlined by fluorescence. Enlarged 3× from ×500.

FIG. 2. Same as Fig. 1. Less well circumscribed perinuclear fluorescence of a distorted lymphoid plasma cell.

anti-macroglobulin, but was subsequently removed during the wash in buffered saline or during PAS procedure. PAS positive clumps in the clear perinuclear area of these distorted cells may represent the material which was originally within the nucleus.

The reason for the observed cellular distortion is not known but the phenomenon has been reported by Louis(5) in peripheral blood smears stained by the Leishman technic following ultraviolet microscopy. Several neutrophils were similarly distorted and it may be, as suggested by Louis, that ultraviolet irradiation is detrimental to cellular structures even after "fixation."

Smears incubated with labeled absorbed anti-human gamma globulin contained cells with an eccentric nucleus and a diffuse cytoplasmic fluorescence similar to that described for plasma cells(6) and myeloma cells(7). The J.W. marrow and carcinoma control marrows contained relatively few of these cells whereas they were numerous in multiple myeloma control smears. No intranuclear fluorescence was seen in anti-human gamma globulin preparations.

Other cellular components of marrow smears had no specific fluorescence with either labeled antiserum. Neutrophilic granules had a faint non-specific fluorescence and other granulocytes, either eosinophils or basophils, contained bright yellow to orange cytoplasmic granules. The background plasma was PAS positive, intensity depending upon thickness of smear. This was consistent with the presence of 8 g % of gamma macroglobulin of high carbohydrate content in the patient's plasma. Plasma fluorescence was concentrated at the red cell surface as noted by Pachter *et al.*(8).

Discussion. The demonstration of immunologic, in addition to histochemical, identity of intranuclear PAS positive material of lymphoid plasma cells and the circulating macroglobulin in a patient with the macroglobulinemia of Waldenström is strong support for the concept that the lymphoid plasma cell contains macroglobulin. Whether the intranuclear material represents macroglobulin formation, storage or destruction and whether this is a normal or abnormal site of macroglobulin metabolism, awaits further study. In view of the analogous situation, wherein myeloma protein has been shown in myeloma cells by the immunofluorescent technic(7) and formed in myeloma tumor by biochemical technics(9), it is reasonable to expect that macroglobulin is formed in lymphoid plasma cells.

At the time of this study, the marrow from patient J.W. contained fewer lymphoid plasma cells than we observed in other patients with macroglobulinemia. Each J.W. bone marrow smear incubated with anti-macroglobulin preparation contained 20 to 30 fluorescent cells and most of these had intranuclear fluorescence. A similar number of fluorescent cells were found in parallel smears incubated with antinormal 6.6 S globulin.

Specific intranuclear fluorescence with the anti-J.W. macroglobulin was demonstrable in cells of one other patient with macroglobulinemia. Recently Curtain(10), using a similar technic, observed cytoplasmic, but not intranuclear, fluorescence of plasmacytoid cells from bone marrow of 2 patients. One of these patients had increased serum gamma macroglobulins but an atypical clinical history for Waldenström's macroglobulinemia(11). The other patient appeared to have multiple myeloma.

Summary. Immunofluorescent and cytochemical technics were used to identify 18S gamma macroglobulin within the nucleus and cytoplasm of lymphocytoid plasma cells from a patient with macroglobulinemia of Waldenström. Intranuclear fluorescence coincided with presence of intranuclear PAS positive protein. This observation supports the concept, suggested by histochemical studies, that circulating macroglobulin and intranuclear protein of lymphocytoid plasma cells are closely related and may be identical. The evidence suggests that macroglobulin is formed in lymphocytoid plasma cells.

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Response of Isolated Auricles to Some Substances of Metabolic Interest.* (25556)

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Inotropic actions of various intermediary metabolites have been studied most often using preparations of rat ventricular muscle (1,2,3), papillary muscle of cat(4) or isolated heart(5,6,7). Similar studies on isolated auricles of rabbit(8) have been conducted in a somewhat different manner and differences in activity noted. Ventricles and auricles also differ with respect to their sensitivity to various inorganic ions(9,10) and exhibit a difference in responsiveness to stimulation of their extrinsic nerve supply(11). These observations led us to perform the following experiments to determine whether or not the auricular effects in the rat of malonic acid and certain intermediates of the tricarboxylic acid cycle are similar to those elicited by these agents in ventricular preparations from the same animal. We also attempted to explain the observed effects utilizing a somewhat different experimental approach than that employed previously.

Materials and methods. Albino rats of Holtzman strain, unselected as to sex and weighing approximately 150 g were used. Animals were anesthetized with ether and the hearts rapidly removed. After excising ventricular muscle, blood vessels, fat and connective tissue, the auricles were suspended in a

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