in 47 cases by the finding of L.E. cells (with necropsy confirmation in seven), in one by typical changes at necropsy, and in one by skin biopsy.

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A SERUM FACTOR IN LUPUS ERYTHEMATOSUS WITH AFFINITY FOR TISSUE NUCLEI

BY

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The L.E. cell phenomenon, first demonstrated by Hargraves *et al.* (1948) in blood from patients with systemic lupus erythematosus, has since received considerable attention. It has been established that the responsible serum factor is present in the gamma-globulin fraction (Lee *et al.*, 1951) and brings about *in vitro* (1) swelling and disintegration of the nuclei of mature polymorphonuclear neutrophils, and (2) phagocytosis of the cell remains by intact granulocytes to form a typical L.E. cell (Rohn and Bond, 1952). Gueft and Laufer (1954), using histochemical techniques, have demonstrated altered nuclear material in the tissues of such patients.

In the present work we have shown that sera giving a positive L.E. cell test contain a globulin factor with affinity for tissue nuclei. These findings are similar to those already briefly reported by Friou (1957) at the 9th International Congress on Rheumatic Diseases.

Method

Tissue and cell preparations were treated with anti-human-globulin sera conjugated with fluorescein isocyanate, and examined microscopically using an ultra-violet light source. Sections pretreated with serum giving a positive L.E. cell test (L.E. positive serum) showed apple-green fluorescent areas where the conjugated antiglobulin serum had reacted with fixed globulin derived from the L.E. positive serum.

Preparation of Tissues.—Human tissues were obtained at operation, or as soon as possible after death. Tissues from

stillborn infants, adult post-mortem tissue, and operation tissue all gave concordant results. Small blocks of unfixed tissue from these sources were instantly frozen in test-tubes in solid CO₂ acetone mixture and transferred to a cryostat cabinet kept at -20° C. in which sections were cut on a microtome at 6 μ thickness and mounted on slides. Each section was covered with a few drops of L.E. positive serum and incubated in a moist chamber for 30 minutes at 37° C. Duplicate sections were similarly treated with normal human serum. After incubation the serum was washed off in buffered saline (pH 7.0) and a drop of the fluorescein-conjugated anti-human-globulin serum placed on each section; after 30 minutes sections were again washed, and mounted in buffered glycerin (pH 7.0).

Preparation of White-cell Films.—The clot from fresh group O blood was broken up with a wooden applicator and the admixture of serum and cells centrifuged in a Wintrobe tube at 3,000 r.p.m. for five minutes. Thin films were then made from the buffy coat and treated in the same way as the tissue sections.

Preparation of Conjugates.—Two rabbit anti-human-globulin sera were used: (1) prepared in rabbits injected intravenously with their own red cells previously agglutinated by pooled human serum (Milgrom *et al.*, 1956); (2) Coombs anti-human-globulin serum. These two sera were conjugated with fluorescein isocyanate according to the method of Coons and Kaplan (1950). Before use the conjugates were absorbed with a dried organ powder prepared from rabbit liver to remove non-specific staining of tissues.

Test Sera.—Three sera giving positive L.E. cell tests were used for pretreatment of tissue sections on white-cell films. Two of these sera were obtained from patients with systemic lupus erythematosus, and the third from a severe and ultimately fatal case of rheumatoid arthritis. Results with all three sera were similar. A number of normal human sera were used as controls.

Examination of Preparations.—Mounted preparations were examined microscopically, using a 250-watt high-pressure mercury-vapour lamp as the source of ultra-violet light. Visible light was absorbed by Wood's filter, and either a quartz condenser or a dark-ground condenser was used. Better definition was obtained with the dark-ground condenser, particularly with higher-power objectives.

Results

Tissues examined were skin, heart muscle, kidney, thyroid, and spleen. Fig. 1 shows a section of kidney which was pretreated with normal serum and then with fluorescent antiglobulin. Another section of the same kidney treated with serum positive in the L.E. cell test, and then with fluorescent antiglobulin, shows specific "staining" of the cell nuclei (Fig. 2). In all tissues examined exposure of the section to "L.E.-cell-test-positive" sera resulted in marked specific fluorescence of the nuclei on subsequent staining with the antiglobulin conjugate. This was never found when normal sera were used to treat the section. The appearance is best shown in tissues containing epithelial cells where the arrangement of nuclei is in orderly patterns of tubules, acini, or strata (Figs. 3 and 4), but was clearly present in every tissue examined. The specificity of staining by the antiglobulin conjugate was confirmed by showing that its ability to stain tissue treated with L.E. positive serum was blocked by pretreatment with unconjugated antiglobulin serum but not by normal serum.

Both the conjugates described above gave similar results, varying only in intensity of fluorescence; some preparations showed brighter fluorescence with one than with the other, but no qualitative difference was observed.

Fig. 5 shows a buffy-coat preparation pretreated with L.E. positive serum and then stained with fluorescent conjugate. The white-cell nuclei fluoresce brightly and specifically. In control films pretreated with normal serum the white-cell nuclei appear as unstained spaces against faint non-specific staining of the cytoplasm. Some granulocytes, possibly eosinophils, show bright non-specific staining of cytoplasmic granules, which serves to emphasize the lack of nuclear staining (Fig. 6).

Positive results were obtained only when sections were cut and treated on the same day. Sections kept for several days before treatment, even when refrigerated, gave variable or negative results.

Discussion

This work shows the presence in the three L.E. positive sera examined of a factor with an affinity for tissue-cell nuclei. This factor was not present in normal sera tested.

FIG. 1.—Kidney cortex. Section pretreated with normal serum, then treated with fluorescent antiglobulin. No nuclear staining. ($\times 160$.)

FIG. 2.—Section from same tissue block as Fig. 1. Pretreatment with L.E. positive serum results in specific staining of nuclei on treatment with fluorescent conjugate. ($\times 160$.)

FIG. 3.—Thyroid gland from neonatal death; section pretreated with normal serum. No nuclear staining on treatment with antiglobulin conjugate. ($\times 160$.)

FIG. 4.—Same tissue as Fig. 3: section pretreated with L.E. positive serum. Bright nuclear staining on treatment with conjugate. ($\times 160$.)

FIG. 5.—Group O blood film, unfixed. Pretreated with L.E. positive serum, then stained with conjugate. Leucocyte nuclei fluoresce brightly. ($\times 480$.)

Previous work has shown that a gamma-globulin fraction is responsible for the L.E. cell phenomenon of Hargraves, a part of which involves alteration of the nuclear material of white cells. This suggests that the L.E. cell factor is of an antibody nature, and the present demonstration in tissue-cell

FIG. 6.—Non-specific fluorescence of cytoplasmic granules in certain leucocytes (? eosinophils). Unfixed blood film pre-treated with normal serum, then stained with conjugate. Nuclei remain non-fluorescent. ($\times 480$.)

nuclei of a fixed globulin derived from L.E. positive serum adds support to this hypothesis.

The present work also confirms a recent finding of Mellors *et al.* (1957), who described specific localization of fluorescein-conjugated antiglobulin serum in the nuclei

of the white cells in an L.E. cell preparation. It thus seems very likely that the "L.E. factor" active against leucocytes is also the factor reacting with the tissue-cell nuclei reported here.

Further work may show whether the existence in lupus erythematosus of an immunological mechanism involving tissue-cell nuclei can account for the presence of altered nuclear material in this disease.

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VIRUS HEPATITIS WITH FEATURES OF PROLONGED BILE RETENTION

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Atypical manifestations of infectious diseases are difficult to recognize when the causative agent cannot be identified. In epidemics their recognition is facilitated, but sporadic cases are always diagnostic problems. "Virus" hepatitis is a well-recognized clinico-pathological entity, although the causative virus still eludes us. Eppinger (1937) recognized that, in addition to a hepatocellular variety, infective jaundice might take a "cholangitic" form which was characterized by more prolonged, deeper icterus, clinical features of obstructive jaundice, and histologically a liver showing periportal cellular infiltration and bile stasis with little evidence of hepatocellular injury. Watson and Hoffbauer (1946) described the cases of eight patients who were exposed to hepatitis and whose illness began as a typical acute hepatitis, but who showed a prolonged course with features of

obstructive jaundice, and they introduced the term "cholangiolitic hepatitis" for such cases. This complication of acute hepatitis has subsequently been described by others (Saint, 1952; Lipschutz and Capson, 1955; Johnson and Doenges, 1956).

It is the purpose of this paper to review 12 cases of acute "virus" hepatitis showing features of prolonged bile retention, with particular reference to the difficulty in diagnosis, to the management, and to the prognosis.

Clinical Features (Table I)

The seven female and five male patients were aged 13 to 59 years (mean 40 years). Two had been in contact with hepatitis during the preceding two months and two had received blood transfusions three months previously. In the others the jaundice occurred sporadically. The onset was always acute with anorexia, malaise, nausea, and vomiting, and smokers developed a distaste for tobacco. Pain in the liver area was unusual. Following the acute prodromata, jaundice appeared and deepened progressively. After about three weeks pruritus was noted, lasting about three weeks, and two patients showed a generalized maculopapular rash which lasted two to three weeks. The liver was enlarged in seven patients, but tender in only three; in two patients the spleen was palpable. The urine showed bile pigment, and an initial urobilinogen excess was soon followed by absence of urobilinogen. Stools became acholic, but as the disease continued pigment was usually noted from time to time.

After the first three to four weeks the patients felt well, were afebrile, gained weight, and the spleen and liver could no longer be felt. Jaundice, however, deepened and lasted 8 to 29 weeks, with a mean of 13 weeks. In three patients the possibility of extrahepatic biliary obstruction was so strong that exploratory laparotomy was performed, but no bile-duct obstruction could be found. These patients made slow post-operative recoveries, one having a transient episode of liver failure with ascites and impending hepatic coma. All the patients made an eventual complete clinical recovery. In seven patients a cholecystogram or intravenous cholangiogram was performed, and this was normal. The patients have been followed up for one month to six years, no relapses or clinical progression to chronic liver disease being noted.

Biochemical Features (Table II)

The peak serum bilirubin level was reached within eight weeks, and averaged 17 mg. per 100 ml., with a range of 6.6 to 27 mg. per 100 ml.; it then fell fairly sharply and steadily but did not fall below 2 mg. per 100 ml. until about the thirteenth week. The urobilinogen returned to the urine about the eighth week with a range of 4 to 18 weeks. The serum alkaline phosphatase level was raised above 30 units per 100 ml. in only three patients, but showed a tendency to increase with the serum bilirubin level and fell some time after the serum bilirubin level was normal; in two patients it was still raised after one year. Serial changes in serum cholesterol values were not remarkable, but three patients showed an elevation at the onset, and one rose later. The serum albumin concentration was not significantly altered. There was a tendency to hyperglobulinaemia, with a proportionate increase in the α_2 - and β -globulin fractions (Fig. 1), which eventually returned to normal. During the first six to eight weeks the flocculation tests were positive in eight patients but became

FIG. 1.—Case 6. Electrophoretic pattern of the serum proteins, showing an increase in the α_2 - and β -globulin fractions.

flocculation tests were positive in eight patients but became