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Demonstration of Gamma Globulin in Vascular Lesions of Experimental Necrotizing Arteritis in the Rat.* (25185)

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In renal and vascular lesions of human periarteritis nodosa, presumably of antigen-antibody character, gamma globulin has been demonstrated by fluorescence microscopy(1). In a few instances of other human and experimental arteritis of similar origin, gamma globulin has also been demonstrated(2,3). It appeared therefore interesting to look for gamma globulin in the walls of vessels with necrotizing arteritis, which is not considered to be due to immunological causes. Necrotizing arteritis was produced in rats by unilateral nephrectomy followed by administration of DOCA(4). These lesions were examined for presence of gamma globulin(5,6).

Material and methods. Thirty-nine female Sprague-Dawley rats weighing approximately 150 g were divided into 3 groups: *Group 1,* consisting of 16 rats, were subjected to unilateral nephrectomy under nembutal and ether anesthesia. Subsequently they were given 1.5 mg of DOCA† intramuscularly 3 times a week for 5 weeks. The dosage of DOCA was then increased to 2.5 mg daily. One % NaCl was provided for drinking water. The diet consisted of standard pellets. This regimen was continued for varying periods to 7½ months after which the animals were sacrificed. From some animals, heart blood was drawn for determination of serum gamma globulin(7) immediately before death. The animals were weighed at beginning, occasionally during, and at end of experiment. Weight loss and a general deterioration, reflected in ruffled fur, lethargy and loss of appetite, were used as criteria for selecting time of sacrificing. Some rats succumbed spontaneously. *Group 2* consisted of 13 animals treated as Group 1 but sacrificed within the first 5 weeks (before increase in dosage of DOCA). Eight died spontaneously within 7 to 10 days after operation. *Group III* consisted of 10 rats which only had unilateral nephrectomy and received an ordinary diet and tap water. Immediately after death, tissues were removed from all organs except central nervous system and skeleton, and prepared in the following ways: Blocks of tissue were fixed in 10% neutral formalin for preparation of paraffin sections. Blocks from various organs were snap-frozen at −70°C in a dry ice-isopentane mixture, stored at −30°C and sectioned in a cryostat at approximately 4 μ. Both paraffin and cryostat sections were stained by the conventional staining methods. Staining with fluorescein-labelled serum was carried out, generally following the method of Coons(5,6): Rabbits were immunized with rat gamma globulin (RGG)(8) and bled from the heart. Gamma globulin was precipitated out with 18% Na_2SO_4(9), purified and conjugated with fluorescein-isothiocyanate(10,11). Cryostat sections of rat tissues were then stained with this labelled rabbit anti-RGG serum globulin solution. Controls included: (1) testing the system with non-fluoresceinated rabbit immune serum for blocking of the reaction, and (2) testing the system with normal rabbit serum

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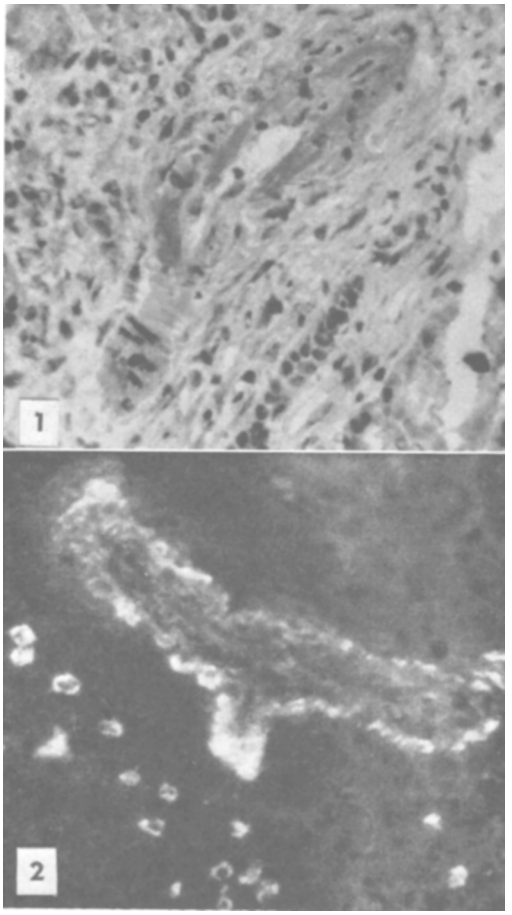

FIG. 1. Small artery in pancreas showing acute necrotizing arteritis with fibrinoid deposits in the media. Cryostat section stained with hematoxylin and eosin.

FIG. 2. Small artery similar to the one shown in Fig. 1. Stained with fluorescein-labelled anti-rat gamma globulin. The fluorescence appears localized chiefly in smooth muscle of media. Plasma cells in environment of involved artery also show fluorescence.

for failure to block the reaction. Fluorescein-stained sections were examined in Zeiss fluorescence microscope using BG 12 filter between light source and specimen, and CG5 filter between specimen and ocular.

Results. Animals in Groups II and III had no demonstrable lesions. In Group I, gross lesions were present in about one-fourth of the animals. Microscopically in large vessels, predominantly splanchnic lesions varied from slight, early swelling of the intima to granuloma formation after 3 months. In the fully developed arteritis, three zones could be recognized: A necrotic inner zone, a granulomatous intermediate zone with radially arranged epithelioid cells and an outer zone of circular collagenous fibres and fibroblasts. Undamaged vessels, when stained with fluorescein-labelled anti-gamma globulin, had no fluorescence. Those blood vessels which had undergone complete necrosis had a diffuse brilliant green fluorescence of the necrotic vessel walls, which was not blocked by the unconjugated anti-rat gamma globulin. The greatest intensity of this fluorescence was in the outermost zone. In those vessels where the structure was still partially preserved (Fig. 1), fluorescence was more focal and could be blocked partially by unconjugated rabbit anti-RGG. The fluorescence was distinctly localized in the damaged smooth muscle (Fig. 2). This was particularly striking where the architecture of the muscle was still recognizable, that is, probably in the milder or earlier phases of the disease process. The smaller the amount of damage in the vessel wall, the more completely could non-specific fluorescence be eliminated. In those arteries presenting a granulomatous reaction, fluorescence was limited to the inner "fibrinoid" zone. This was not entirely specific.

The same degree and extent of fluorescence was noted in small arteries and arterioles, where there was minimal vessel damage, as was noted for the larger arteries.

When sections were stained with anti-rat plasma serum from which the anti-rat globulin had been precipitated out, no fluorescence was obtained in the damaged vessel wall, regardless of degree of necrosis.

In kidneys, besides necrotizing angiitis, lesions were observed in many glomeruli which were extensive but varied in severity and resembled those seen in malignant hypertension in man. The mildest change consisted of increased cellularity of the glomerulus and thickening of the glomerular tuft with hyalinization; the severe change, of partial or complete necrosis of the glomerulus. The proximal convoluted tubules were dilated and filled with large protein casts. In the early glomerular lesions, gamma globulin could be demonstrated in thin, wavy, fragmented fluorescent lines, apparently corresponding to the base-

ment membrane. In more advanced lesions, gamma globulin was also seen in many of the swollen endothelial cells as well as in the precipitated protein within the capillary lumen and in Bowman's space.

In animals which survived 7½ months, and which had not received DOCA for the last 3½ months, no active vascular lesions were found. In only a few of the thickened vessel walls was gamma globulin still present. In the kidney focal glomerulo-fibrosis and cystic dilatation of the tubules without gamma globulin were noted.

Discussion. Localization of specific fluorescence in the vessel wall suggests binding of rat gamma globulin in the damaged smooth muscle. Control experiments with anti-rat plasma rabbit serum from which anti-rat gamma globulin had been removed yielded no specific fluorescence in the necrotic vessels. This would suggest that rat gamma globulin is selectively deposited in the wall of the damaged vessel and that this is not the result of simple diffusion of plasma constituents from the circulating blood into the tissues.

The observation by others of fibrin in vascular lesions of human peri-arteritis nodosa (12) and of serum albumin along with gamma globulin in vascular lesions of experimental serum sickness(3) should caution against hasty conclusions as to the significance of these findings.

In the kidney on the other hand, increased amount of gamma globulin in the more damaged glomeruli may merely reflect altered permeability secondary to severe capillary damage.

Summary. Experimental necrotizing arteritis was produced in female Sprague-Dawley rats by administration of large doses of DOCA and NaCl after unilateral nephrectomy. Presence of RGG in damaged vessel walls was demonstrated by fluorescence antibody technic of Coons. Gamma globulin is deposited in smooth muscle of damaged small arteries, especially in early phases of vascular damage, suggesting that antibody may not be required for this process.

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Microfluorimetric Evidence of Antigenic Difference Between *Entamoeba histolytica* and *Entamoeba hartmanni*. (25186)

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The complex of amebae known as *Entamoeba histolytica* includes: a large hematophagous form (20-40 μ diameter) associated with acute amebic dysentery; a medium sized form (10-20 μ) generally found in asymptomatic carriers; and a small organism (5-11 μ) generally regarded as never associated with disease(1,2). Burrows(3) recently reviewed the literature dealing with so-called "small race" and, in addition, contributed new morphological data comparing small and medium sized amebae. He concluded that the "small race" was properly a different species, and that its original designation of *Entamoeba*