We have not been able to obtain CPE with the Albany strains, types 4 and 6. Slater and Syverton(1) obtained propagation with a type 4 (Minnesota strain) virus for 24 consecutive passages in minced mouse embryonic cells. We have not obtained either CPE. or evidence of virus multiplication on repassage of tissue culture fluids in suckling mice with the Albany type 4 virus. Lehmann-Grube and Syverton(6) also reported that type 6 propagated in human amnion cells. We failed to obtain signs of virus growth in human amnionic cells. although at the 4th passage fluid overlays obtained from cultures inoculated with this serotype produced typical disease in mice. When cultural conditions are properly fulfilled types not yet associated with CPE will probably yield. Since the media used for growth and maintenance of cells in various laboratories often differ. measurements on susceptibility and resistance of cultured cells for Group A Coxsackie viruses in different laboratories will not always be the same.

In previous reports(6,7) several Group A viruses after serial propagation in human amnion cells were, in comparison with infected mouse tissues from which they were derived, associated with a significant reduction in virulence for mice. Our results support these earlier findings. Indeed. undiluted tissue culture fluids have been required to produce illness in a fraction of infected mice. These observations point up the unreliability of using suckling mice as indicator of virus multiplication in tissue cultures. Viral antigens present in infected cells may however be delineated by the fluorescent antibody staining method(11).

Summary. Seventeen serotypic Group A Coxsackie viruses, representing prototypes established by Dalldorf and Sickles(5,10) have been propagated in tissue cultures. Sixteen strains produced CPE in primary human amnion cells; another (type 7) which has failed thus far to affect amnion cells produced CPE in renal cells obtained from monkeys. Among these 17 Group A viruses were 5 serotypes heretofore not associated with CPE in tissue cultures.

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Electron Microscopy of the Arthus Reaction, Using Ferritin as Antigen. (26683)

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Although it is known that the Arthus phenomenon is dependent upon presence of circulating precipitating antibody(1,2,3) and that antigen is localized in blood vessel walls (4), the antigen-antibody complex has heretofore not been demonstrated in the reactive site.

The use of the electron-dense protein, ferritin, as antigen(5,6) has enabled direct visualization of the antigen-antibody precipi-

tate in the active Arthus lesion.

Materials and methods. 1. Method of immunization. Horse spleen ferritin, Nutritional Biochemicals, Cleveland, Ohio, prepared by the method of Grannick(7) was used for production of Arthus sensitivity in 4-6-month-old Hartley Strain male guinea pigs and 4-6-month-old albino male rabbits.

Ten cc of a 0.1% ferritin in normal saline solution was emulsified with 10 cc of Complete Adjuvant (Freund), Difco Labs, Detroit, Mich. Rabbits were initially injected with 0.5 cc of emulsion in each of 4 subcutaneous sites. Fourteen days later they received subcutaneous injections of 2 cc of an emulsion of incomplete Adjuvant (Freund), Difco Labs, and ferritin.

Guinea pigs were immunized by intradermal injection of 1 cc of the Complete Adjuvant-Ferritin emulsion in divided doses into 8 sites in the nuchal region. Three weeks later similar injections were given using 1 cc of Incomplete Adjuvant-Ferritin emulsion.

2. Determination of specificity of immune globulin for ferritin. The animals were bled prior to, and at varying times during the sensitization, and when the lesions were biopsied. Immune and non-immune globulin was obtained by ammonium sulphate fractionation.

Immunoelectrophoresis(8) was performed to test the immunological specificity of the ferritin-antiferritin reaction. Precipitation arcs of similar contour and mobility correspinding to serum alpha globulins developed between ferritin and immune rabbit globulin and between ferritin and immune guinea pig globulin. No precipitation line formed between normal rabbit globulin and ferritin.

3. Electron microscopy of *in vitro* ferritin-antibody precipitates. After 24 hours, the 2 precipitin lines obtained by immunoelectrophoresis were cut from the agar gel and fixed, dehydrated and embedded in methacrylate for electron microscopy.

Precipitates of ferritin and immune globulin obtained in the equivalence zone were centrifuged into a pellet and prepared for electron microscopy.

In addition precipitates of ferritin and immune globulin were obtained on grids by drying a dilute solution of ferritin on a grid and then superimposing one drop of immune globulin for 1-3 minutes.

4. Production of Arthus reactions. Arthus reactions were produced in skin and skeletal muscle of sensitized animals by intradermal or intramuscular injection of 0.1 cc-0.2 cc ferritin solution. Unsensitized animals were similarly injected with ferritin.

5. Electron-Microscopy of Arthus lesions. Active Arthus lesions induced in skin and muscle were biopsied 15 minutes, 60 minutes, 3½ hours, 12 hours, and 24 hours after injection of ferritin. Control biopsies were performed at the same intervals on skin and muscle of non-sensitized animals injected with 0.1 cc-0.2 cc ferritin solution. Half of each specimen was fixed in 10% buffered formalin for light microscopy. Blocks from the remaining tissue were fixed immediately for electron microscopy in Palade's osmium tetroxide buffered fixative(9). The skin was fixed for 2 hours and the muscle for 3½ hours. The tissue was then dehydrated in a graded series of alcohols, and embedded in 1:5 methyl-butyl methacrylate. Sections were cut on a Porter-Blum ultramicrotome, and then mounted on formvar-coated grids. The sections were examined with an RCA EMU 3B electron microscope.

Results. 1. Light microscopy of Arthus lesions. Typical Arthus reactions developed in the skin and skeletal muscle of sensitized rabbits and guinea pigs when challenged with ferritin. The inflammatory response progressed rapidly reaching greatest intensity within 6-8 hours. Control animals showed neither gross nor microscopic evidence of a reaction.

The vascular inflammation, thrombosis of small blood vessels, inflammatory exudate and tissue necrosis were typical of the histologic changes of active Arthus lesions(10). Small numbers of acute inflammatory cells were seen in the walls of small blood vessels in 15 minutes. The ferritin-induced reactions could not be differentiated grossly or histologically from those obtained in animals sensitized to ovalbumin. The histologic reactions in Arthus lesions produced in skeletal muscle were similar to those observed in skin.

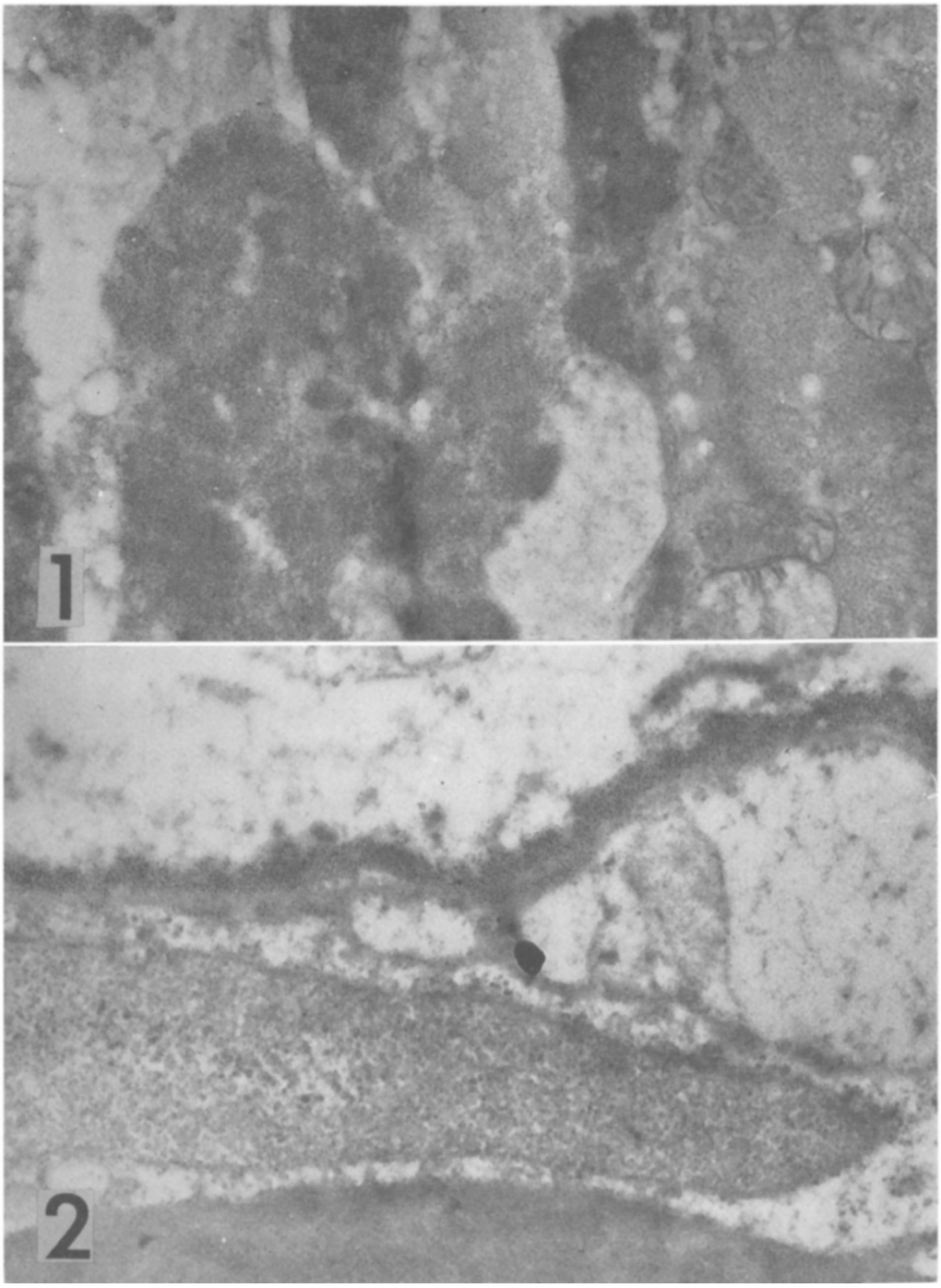

FIG. 1. Electron micrograph of Arthus reaction in guinea pig skeletal muscle. Precipitated ferritin molecules in interstitial tissue adjacent to striated muscle—3½ hr after challenge with ferritin. Magnification × 18,225.

FIG. 2. Electron micrograph of Arthus reaction in guinea pig skeletal muscle. Aggregated ferritin molecules are lined up alongside and in the basement membrane of a small blood vessel —3½ hr after challenge with ferritin. Magnification × 36,675.

2. Electron microscopic appearance of ferritin-antibody precipitates. Ferritin dried from a dilute solution on a formvar-coated grid appeared as randomly distributed discrete granules. The electron microscopic appearance of the ferritin-antibody precipitates were similar to those reported by Easty (11). The electron opaque granules were clumped and enveloped by amorphous material of lesser density, presumed to be antibody.

In control preparations of ferritin and nonimmune globulin, enveloping material was rarely seen.

3. Electron microscopic appearance of Arthus lesions. Aggregated ferritin molecules associated with an amorphous material of lesser density, comparable to *in vitro* ferritin-antibody complexes were constantly seen in Arthus lesions (Fig. 1).

The complexes were not more concentrated in the vicinity of small blood vessels than elsewhere. Nevertheless they had a distinctive circumferential distribution about the basement membranes of the vessels (Fig. 2). In some vessels ferritin aggregates were seen on either side of basement membranes and in the underlying endothelial cells.

The ferritin-antibody complex was seen in the vessels and adjacent structures of Arthus lesions biopsied as early as 15 minutes and up to 24 hours subsequent to challenge with antigen. Hemorrhage and necrosis were apparent at 3½ hours. The electron microscopic evidence of progressive tissue necrosis paralleled that seen in light microscope sections. Large aggregates of ferritin were seen within and between necrotic muscle fibers, and in the cytoplasm of cells, and mixed with erythrocytes and necrotic debris.

In control specimens there was no tissue damage. Ferritin molecules were difficult to locate in these even when biopsied 15 minutes after injection of antigen. When ferritin was seen it was scattered randomly throughout the sections and showed no localization about basement membranes of small vessels. Aggregated ferritin molecules surrounded by material of lesser electron density were not seen in control sections.

Discussion. The altered electron microscopic appearance of ferritin in Arthus lesions indicates that the antigen was precipitated by a protein material. The ferritin-protein complex closely resembled the *in vitro* ferritin-antibody precipitates. In view of the fact that precipitins were present in the circulating blood of the animals, it may be inferred that the ferritin-protein masses were in fact antigen-antibody complexes.

Other investigators (12) employing immunofluorescent technics have demonstrated that antigen is localized in the subendothelial region of blood vessels in the Arthus reaction. The findings in the present study differ somewhat in that the antigen-antibody complexes were found not only in the vessels but also in adjacent tissues.

The circumferential arrangement of ferritin-antibody complexes in and about the basement membranes of small blood vessels suggests that this site of localization is a prelude to the vascular necrosis which is such a prominent histological feature of the reaction.

The prominence of ferritin-antibody complexes in interstitial tissue, as compared to the minimal interstitial fluorescence of antigen in the immunofluorescent technics, can be accounted for by differences in the methods employed.

In the former instance all the antigen and antibody which react to cause the Arthus lesion is visible, whereas in the latter only the unbound antigen or antibody is available for fluorescence.

Summary. Arthus lesions were produced in skin and striated muscle of guinea pigs and rabbits sensitized to ferritin. Biopsies of these lesions were then examined with the electron microscope. Electron micrographs of lesions obtained as early as 15 minutes following challenge with antigen demonstrated ferritin surrounded by amorphous material of lesser electron-density comparable to *in vitro* ferritin-antibody precipitates. Ferritin-antibody precipitates were widely distributed throughout the lesions and were preferentially localized in and on either side of the basement membranes of small blood vessels.

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Tetracycline Accelerates Elimination of Strontium from Bones in Mice.* (26684)

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The antibiotic tetracycline, identified in tissues by its golden fluorescence, is preferentially deposited in growing bone(1), and has been shown to inhibit skeletal formation in the chick embryo(2). It has been hypothesized that the tetracyclines interfere with cellular and enzymatic processes by virtue of their affinity for inorganic cations, especially calcium and magnesium(3). We have investigated the effects of tetracycline on strontium metabolism in adult mice, using Sr-85, a gamma emitter, as tracer. Aside from its importance in radioactive fallout (as the isotope Sr-90), strontium has gained increasing attention as a tracer for studying bone metabolism, being handled in the body qualitatively similarly to calcium(4).

In this experiment, 19 Swiss Webster mice were given single injections of $Sr^{85}(NO_3)_2$, 1 μc each, specific activity 200 μc/mg. The control group, consisting of 10 animals, received no other treatment. The 9 mice in the second group were given daily injections of tetracycline, 150 mg/kg, starting on the 4th day prior to Sr-85 injection, and continuing to the 25th day after Sr-85 injection, when all the animals were sacrificed. The amount of Sr-85 in each mouse each day was measured with an external gamma detector (NaI scintillation counter). Average values of total body Sr-85 against time are shown in Fig. 1. Each curve has a fast component, representing direct excretion from the body of a portion of the initial dose, and a slower component, representing gradual elimination of the portion incorporated into bone. Extrapolation of the slow exponential yields the fraction of the original dose which was deposited in bone. At autopsy, no significant

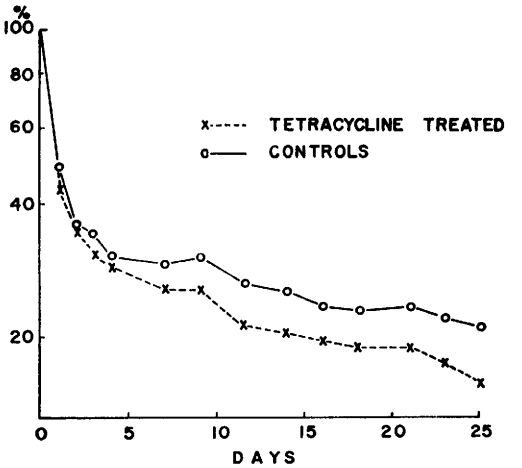

FIG. 1. Turnover of Sr^{85} in tetracycline treated mice.

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