ing tumor cells *in vitro*, or by application of X-ray, and Gerber(11) demonstrated infectious SV_{40} virus in sensitive indicator cells cultivated *in vitro* in contact with SV_{40}-induced hamster ependymoma cells which were free of detectable virus. The state of the virus in the cell which is essential to or associated with continued malignancy and the state of the virus in the cell which can be caused to produce infectious virus are unknown and they may be quite different. Thus, capacity to yield infectious virus might be only an incidental property of certain of the cells which are carrying the virus in a "latent" state contrasted to a more "integrated" form of viral nucleic acid essential to expression of cancer. Such difference in virus state might explain why some tumor lines yield virus and others do not.

Summary. Findings relative to the pathogenesis of SV_{40} virus in inducing tumor in hamsters are discussed. Infectious virus disappeared rapidly from the animal and was not demonstrable until its appearance in tumor. The quantity of virus was related to tumor size. Infectious virus was commonly demonstrable in tumor but was not always present and appeared not to be essential to retention of oncogenic quality. Initial neutralizing antibody response in a portion of animals to inoculated SV_{40} virus appeared unrelated to subsequent development or failure to develop tumor.

The technical assistance of W. Raupp and T. Beddow is gratefully acknowledged.

1. Sweet, B. H., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 420.
2. Girardi, A. J., Sweet, B. H., Slotnick, V. B., Hilleman, M. R., *ibid.*, 1962, v109, 649.
3. Girardi, A. J., Sweet, B. H., Hilleman, M. R., *ibid.*, 1963, v112, 662.
4. Almeida, J. D., *Canad. Med. Assn. J.*, 1963, v89, 787.
5. Hamparian, V. V., Hilleman, M. R., Ketler, A., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 1040.
6. Black, P. H., Rowe, W. P., *J. Nat. Cancer Inst.*, 1964, v32, 253.
7. Black, P. H., Rowe, W. P., Turner, H. C., Huebner, R. J., *Proc. Nat. Acad. Sci.*, 1963, v50, 1148.
8. Black, P. H., Rowe, W. P., *ibid.*, 1963, v50, 606.
9. Sabin, A. B., Koch, M. A., *ibid.*, 1963, v50, 407.
10. ———, *ibid.*, 1963, v49, 304.
11. Gerber, P., *Science*, 1963, v140, 889.

Received March 31, 1964. P.S.E.B.M., 1964, v116.

Immunofluorescent Study of Experimental *Trypanosoma cruzi* Infection.* (29356)

ERVIN ESSENFELD AND ROBERT H. FENNELL, JR. (Introduced by E. Farber)
Department of Pathology, University of Pittsburgh School of Medicine and Presbyterian-University Hospital, Pittsburgh, Pa.

The fluorescent antibody technique has been utilized in the identification of organisms ranging in size from viruses to protozoa (1). Two techniques may be employed, the direct and indirect. The direct technique involves application of a conjugate containing the antibody to the smear or section; the indirect technique involves application of the specific antibody which when bound to the antigen is identified by staining with a conjugate containing an antibody against the specific antibody. The indirect technique has the advantages of being more sensitive and offering a mechanism for serologic testing.

The presence of antibodies in a given serum is demonstrated by applying the test serum to a known antigen. The excess serum is washed away and a fluorescein-conjugated antibody to the test serum is applied. If an antibody to the known antigen is present in the test serum, the antigen will be stained, but otherwise it will remain unstained when examined in fluorescent light. This method of testing has been used to identify antibodies to various organisms including viruses and bacteria(1-2).

* Aided by U.S.P.H.S. grant.

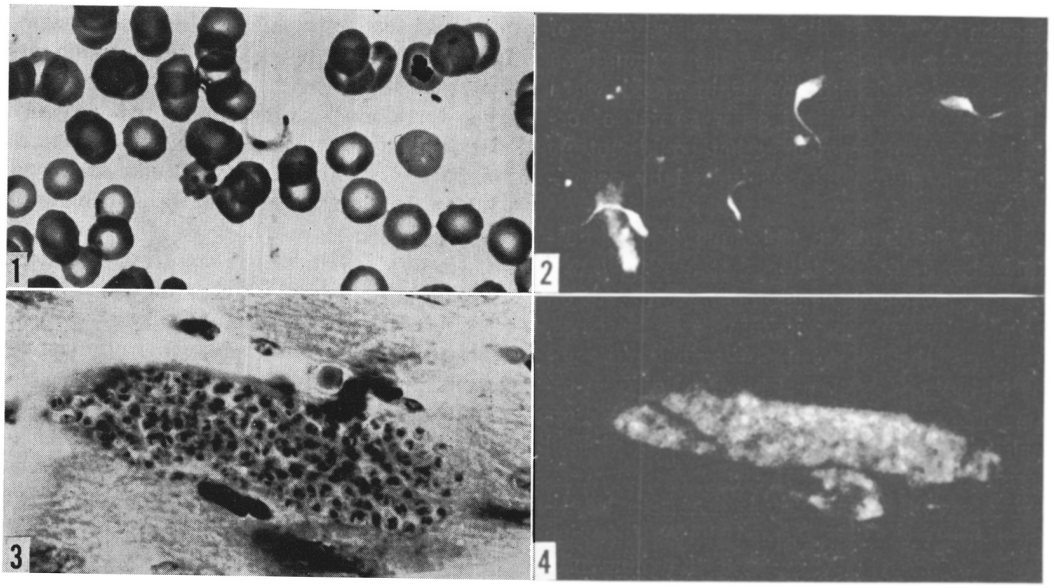

FIG. 1. *Trypanosoma cruzi* present in guinea pig blood smear (see arrow). Wright stain (× 970)

FIG. 2. Immunofluorescent preparation showing specific staining of the trypanosoma form (blood smear). (× 970)

FIG. 3. Nidus of leishmania forms of *Trypanosoma cruzi* in guinea pig myocardium (H & E stain). (× 970)

FIG. 4. Immunofluorescent preparation shows specific staining of the leishmania form of *Trypanosoma cruzi* in guinea pig myocardium. (× 970)

The present study was done to determine if *Trypanosoma cruzi* could be identified in both the blood trypanosomal and the tissue leishmanial forms by the fluorescent antibody technique. It was found that guinea pigs infected with *Trypanosoma cruzi* developed an antibody as evidenced by the successful staining with conjugated convalescent serum of both its forms and there was cross-immunity between the 5 stains of *Trypanosoma cruzi* used in this study.

Materials and methods. Adult guinea pigs of both sexes were inoculated by subcutaneous injection of *Trypanosoma cruzi* obtained from Dr. Carlos Kozma of Caracas, Venezuela. Smears of peripheral blood were examined at weekly intervals to establish that infection occurred (Fig. 1). After parasitemia subsided animals were bled by cardiac puncture. The whole serum thus obtained was conjugated to fluorescein isothiocynate and used as a stain for *T. cruzi* in smears and sections. Animals were sacrificed at varying times during the acute and latent phases of the infection. Representative tissues including heart, lung, spleen, kidney, and other organs were fixed in formalin for routine sectioning and frozen in liquid nitrogen for fluorescent antibody studies. The frozen tissues were stored at minus 20°C and sections were cut in a cryostat. Staining and examination of tissues were by technique as previously described(1).

Anti-guinea-pig globulin obtained commercially (Hyland Laboratories, Los Angeles, Calif., Lot RP#41862) was conjugated to fluorescein isothiocynate and used to identify guinea pig globulin in the indirect technique.

Attempts to identify an antibody in guinea pig serum using agar-gel diffusion with suspensions or extracts of *T. cruzi* were unsuccessful.

Results. The fluorescein conjugated convalescent guinea pig serum when applied as a stain to smears of peripheral blood stains the trypanosoma forms demonstrating details of body structure quite clearly (Fig. 1, 2). In sections of heart muscle it was possible to demonstrate nests of leishmania with the same conjugates (Fig. 3, 4). There was cross

reaction between the various strains of *Trypanosoma cruzi* in that the serum from animals infected with one strain would, when tested, show staining of other strains of organisms. Also, cross infection could not be induced in guinea pigs as judged by parasitemia.

Using the indirect technique it was possible also to demonstrate that antibody was present. When test serum was placed on smears known to contain trypanosomes, staining with fluorescent anti-guinea pig gamma globulin yielded positive results. The serum from animals not infected, that is normal guinea pig serum, when so tested yielded negative results.

Discussion. This study indicates that antibody to *T. cruzi* is formed during infection in the guinea pig. It suggests that immunity is in part related to a circulating antibody. Cross-reaction between the 5 strains tested is to be expected as reported by others(3,4,5).

Convalescent serum conjugated to fluorescein may be useful in identification of organisms in smears and in tissue sections as both trypanosoma and leishmania forms can be stained. The fluorescent antibody technique may offer an additional tool in investigation of the pathogenesis of human Chagas' Disease. It may be that antigenic remnants that persist in the heart can be identified. This would furnish circumstantial evidence that although immune mechanisms are operative in the pathogenesis of the late stages of Chagas' Disease it need not be on an autoimmune basis.

Summary. Guinea pigs were infected with *Trypanosoma cruzi.* Five different strains were used and cross-immunity was evident between these strains. Serum from convalescent guinea pigs conjugated with fluorescein could be used as a stain for both the blood trypanosoma form and the tissue leishmanial form. Antibodies against the forms could also be identified using the indirect technique.

1. Beutner, E. H., *Bacteriol. Rev.*, 1961, v25, 49.
2. Fife, E. H., Jr., Muschel, L. H., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 540.
3. Hauschka, T., Goodwin, M. B., Palmquist, J., Brown, E., *Trop. Med.*, 1950, v30, 1.
4. Kagan, I., Norman, L., *J. Infect. Dis.*, 1961, v108, 213.
5. Norman, L., Kagan, I., *ibid.*, 1960, v107, 168.

Received April 1, 1964. P.S.E.B.M., 1964, v116.

Conversion of Linoleic-1-C^{14} Acid to Arachidonic Acid in the Gerbil. (29357)

SAMUEL GORDON* AND JAMES F. MEAD
Department of Biophysics and Nuclear Medicine, School of Medicine, University of California at Los Angeles

Relatively high levels of arachidonic acid have been found in rat plasma cholesterol esters by Howton and Hashimoto(2). Swell and associates(3) have confirmed this, and in a comparative study of the serum cholesterol ester fatty acid (CEFA) of 8 species of animals, suggested an inverse relationship between susceptibility to atherosclerosis and the arachidonic acid content of the cholesterol ester fatty acids. In the rat, which is relatively resistant to the production of atherosclerosis, the CEFA arachidonic acid content is very high (50%), whereas those species, *e.g.*, chicken, rabbit, pig, and guinea pig, whose CEFA contained relatively little arachidonic acid (1-2%) were readily susceptible to atherosclerosis.

Since the gerbil has been found to be resistant to atheroma formation(4), it might be expected on the basis of the above correla-

* This study was done during a leave of absence from Biochemical Research Section, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.; and supported in part by contract between Atomic Energy Commission and Univ. of California at Los Angeles.