(3) suggest that since tissue oxygen tension is less in the subendocardium of the dog than in the subepicardial layer, greater oxygen lack may be encountered in the thicker left ventricle than in the right ventricle. They suggested that under conditions of exercise the inner regions may meet part of their energy requirements by anaerobic mechanisms. In the present experiment the greater workloads encountered by the left ventricle possibly increased the anaerobic demands of this tissue and therefore produced the interventricular differences seen in Fig. 1 and 2.

Summary. Graded cardiac work loads were imposed on fasted rats by varying the severity of exercise in acute experiments. Trichloroacetic acid soluble (TCA) and residual glycogen fractions were determined in both ventricles. Glycogen depletion varied with the severity of the exercise and was produced almost exclusively in the TCA fraction. Left ventricular TCA glycogen was affected more than that of the right ventricle.

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Received July 14, 1965. P.S.E.B.M., 1966, v121.

Multiple Virus Infection of Monkey Kidney Cells in Culture.* (30831)

G. D. HSIUNG,† T. ATOYNATAN,† AND L. GLUCK
Department of Epidemiology and Public Health and Department of Pediatrics, Yale University School of Medicine, New Haven, Conn.

Infection of a single animal cell with 2 viruses has been studied previously(1,2,3,4,5), often with inclusions localized in different intracellular sites. For example, a single cell infected with both herpes simplex and vaccinia viruses showed herpes intranuclear inclusions and vaccinia intracytoplasmic inclusions. This report describes a double infection with SV40 and measles virus occurring in the same nucleus of single cells. In addition, these doubly infected cells were highly susceptible to superinfection with poliovirus.

Materials and methods. Virus strains. SV40 and measles virus were isolated from cell cultures prepared from the kidneys of a sick green monkey. SV5 was isolated from rhesus monkey kidney cultures during a simian virus surveillance study(6). Poliovirus type 1, LSC strain, was used in the interference experiments.

Tissue cultures. Primary rhesus or green monkey kidney tissues were trypsinized in the usual manner(7). Roller tubes or Leighton tubes containing 11 × 22 mm coverslips each were seeded with 1-2 ml of a cell suspension containing 3×10^5 cells per ml. These cultures were incubated at 37°C in a stationary position and complete cell monolayers were obtained 6-7 days after seeding.

Staining cultures. Infected cultures on coverslips were washed in phosphate buffered saline and fixed in Zenker's acetic acid solution for hematoxylin-eosin (H&E) staining. For the Feulgen reaction, or for acridine orange fluorescent preparations, Carnoy's fixative fluid was employed. The acridine orange fluorescent preparation described previously by Gluck and Kulovich(8) was used with some modification. After rapid hydration in 80, 70, and 50% alcohols, the coverslips with infected cells were rinsed in 0.002 M $MgSO_4$ solution. Then they were stained for 3 minutes in 0.01% acridine orange in 0.067 M Na_2HPO_4 and KH_2PO_4 buffer of pH 8.0.

* Supported by USPHS Research Grant AI-05313 of Inst. of Allergy & Infect. Dis., N.I.H.

† Present address: Dept. of Medicine, New York University School of Med., New York City.

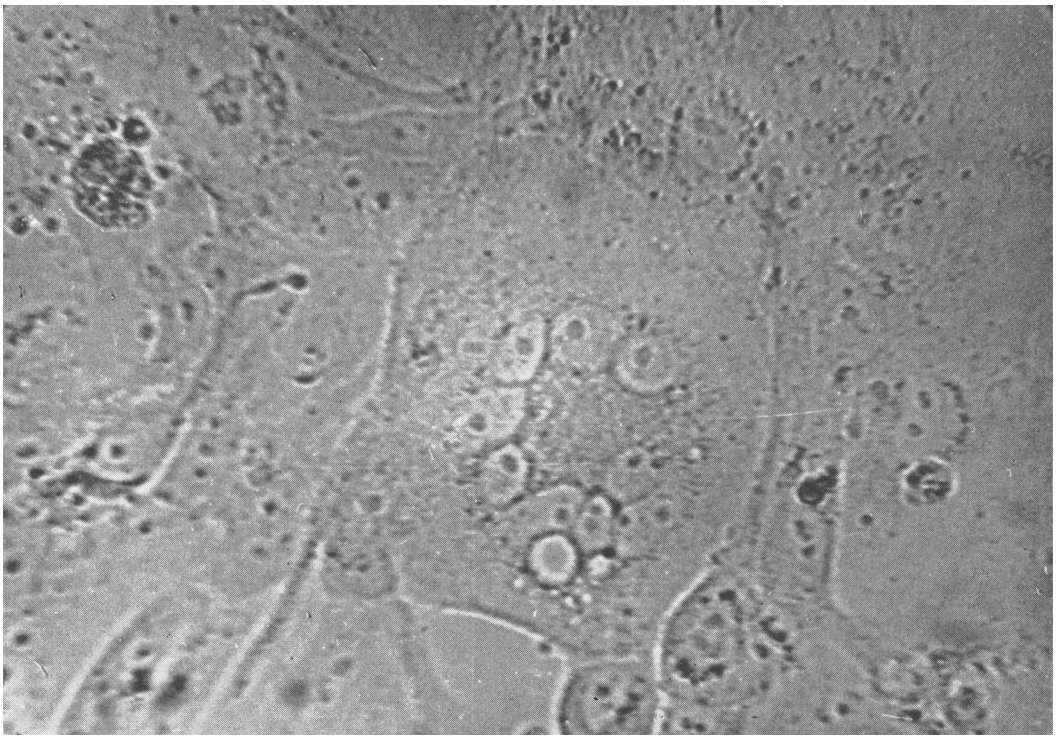

FIG. 1. Cytopathic effect produced by measles virus and SV40 in green monkey kidney cell cultures 28 days after seeding (490 ×).

Excess dye was removed by rinsing in 0.067 M phosphate-0.002 M $MgSO_4$ buffer of pH 8.0. Finally, the stained preparation was differentiated in 0.1 M $CaCl_2$-0.002 M $MgSO_4$ solution, washed in phosphate buffer and mounted in a drop of the same buffer. Microscopic examinations were made immediately using an ultraviolet light source.

Sources of type specific antisera. SV40 and SV5 antisera were prepared in rabbits. Measles antiserum was prepared in monkeys. All antisera were purchased from commercial sources. Paired human sera of pre- and post-measles vaccination were obtained through the courtesy of Dr. F. L. Black.

Neutralization test. Sets of serial 10-fold dilutions of virus suspensions were each mixed with equal volumes of 2-fold dilutions of antiserum, and incubated at room temperature for one hour. The mixtures then were inoculated into culture tubes. Cytopathology in stained preparations and cytopathic effects (CPE) in fluid cultures were used for determination of measles and SV40 virus infectivity titers. For SV5, hemadsorption-inhibition method was used.

Results. Recognition of naturally acquired double infection of monkey kidney cells. A green monkey *(Cercopithecus aethiops)*, which arrived in New Haven on October 23, 1963, appeared to be sick. On November 5, the animal was sacrificed and the kidneys were removed for culture. On December 2, syncytial cells were seen throughout the cultures (Fig. 1). In H&E and acridine orange stained preparations, two distinct kinds of intranuclear inclusions were observed. Neutralization tests using paired hyperimmune antiserum for SV40 and paired human sera for measles virus confirmed the identity of each virus. Antiserum prepared in monkeys for measles virus was not satisfactory for this experiment, since both measles and SV40 viruses were neutralized by the same serum sample.

When SV40 and measles virus intranuclear inclusions occurred in separate cells (Fig. 2), or when a single cell showed inclusions of the

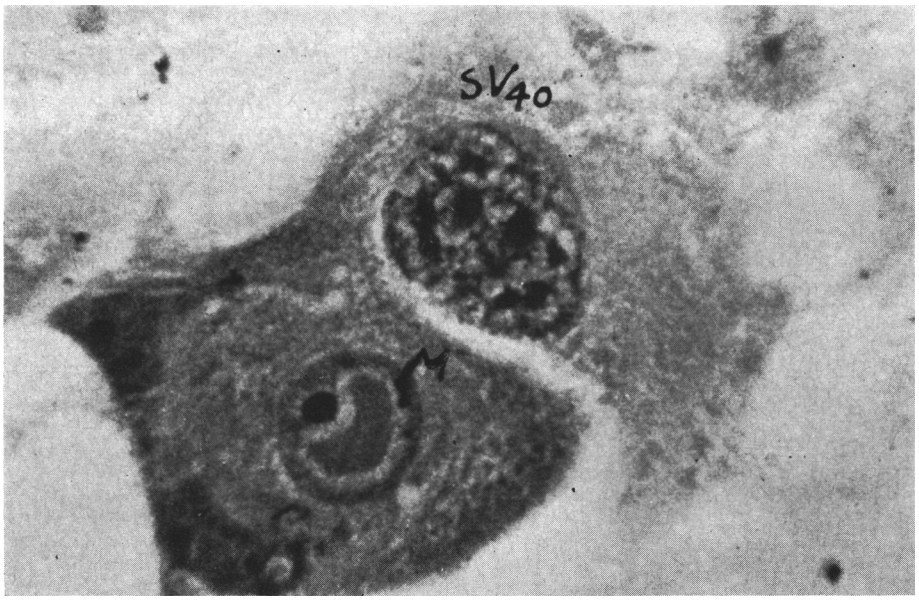

FIG. 2. Measles virus (M) and SV40 intranuclear inclusions. H&E stained preparation 1600 ×.

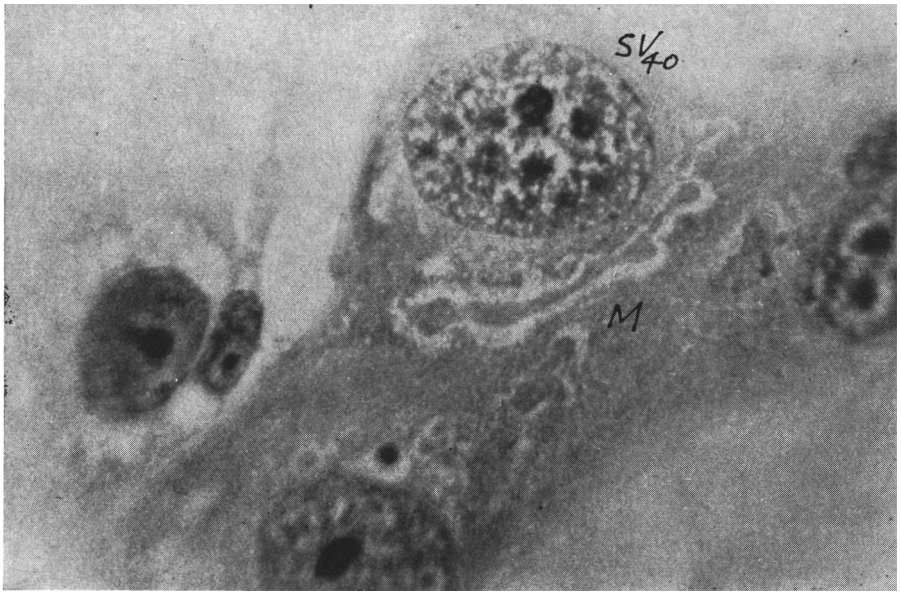

FIG. 3. Measles virus (M) intracytoplasmic inclusion and SV40 intranuclear inclusion. H&E stained preparation 1600 ×.

2 viruses at different sites (Fig. 3), the type of virus infection was not difficult to recognize. In H&E stained preparations, SV40 intranuclear inclusions were basophilic and purplish-blue in color; whereas, measles intranuclear inclusions were eosinophilic and appeared pinkish. However, it was difficult to recognize a doubly infected nucleus containing inclusions of both virus types. The latter was achieved by the modified method of the acridine orange staining technique using phosphate buffer at pH 8.0 instead of at a low pH. The doubly infected nuclei appeared reddish brown fluorescent; whereas, SV40 inclusions were brilliant green. When the doubly infected cultures were exposed to

Feulgen reaction, bright red Feulgen positive intranuclear inclusions of SV40 were observed. No measles virus inclusions, either intranuclear or intracytoplasmic, were seen using the Feulgen preparations.

Poliovirus challenge in cultures infected with one, two or three viruses. A mixture of measles and SV40 stock virus was inoculated into cultures of rhesus monkey kidney cells infected with SV5 virus. Intracytoplasmic inclusions of measles virus, and intranuclear inclusions of measles or SV40 were seen throughout the SV5 infected cultures. SV5 was evidenced by the hemadsorption of guinea pig erythrocytes to the culture cells. An experiment was designed to investigate differences in poliovirus yield upon challenge of cultures infected with one or more viruses. This was done by determination of growth of poliovirus type 1 in rhesus cultures previously infected with other viruses. Prior to inoculation of poliovirus, rhesus cultures were infected with SV5 for 3 days, SV40 for 7 days, and measles for 14 days. These triply infected cultures were then superinfected with poliovirus type 1. At the time the poliovirus was added, the titers of SV5, SV40 and measles were approximately 6.0, 5.0, and 3.5 log TCD_{50} per ml, respectively. Twenty-four hours after challenge, typical poliovirus CPE was observed. There was no recognizable difference or delay in CPE when comparing cultures infected with 2, 3 or 4 kinds of viruses and those infected with poliovirus alone. In addition, poliovirus titers, as determined by 2 separate experiments, were essentially the same whether or not the cultures had been previously infected.

Separation of virus mixtures. A suspension containing SV5, SV40 and measles virus was subjected to ultrafiltration through a millipore filter. SV40 passed through a 100 mμ filter and to some degree through a 50 mμ filter, but measles and SV5 viruses were retained by the 100 mμ filter. It was therefore easy to separate SV5 and measles virus from SV40. When SV5 and measles virus were treated with diethyl ether, both virus titers were reduced; although measles virus was somewhat less sensitive to the ether treatment than SV5. Furthermore, measles antiserum prepared in monkeys also contained antibody to SV5 as well as to SV40, and such serum could not be used for separation of these viruses.

Discussion. As the laboratory techniques improved, recognition of multiple virus infections in tissue culture systems becomes more frequent. The observation in this report represents only one example of mixed virus infection which may occur in primary cultures used for various purposes. It is, therefore, suggested that routine cultures should be examined carefully for the existence of such endogenous contaminants.

It was reported previously both by this laboratory and by others(4,9,10) that measles, SV40 or SV5 did not interfere with the multiplication of poliovirus. Nor did the presence of such extensive "inapparent" infections with 2 or 3 viruses in primary monkey culture affect the yield of poliovirus type 1. As shown by the present experiments, the "stock" obtained from such cultures would be inadvertently contaminated with these viruses.

That the measles antiserum prepared in monkeys showed neutralizing capacity to SV5 and SV40 was not surprising since most of the monkeys were probably infected with these viruses(6). Thus, prior to immunization of the animals, it would be advisable to have the serum sample tested against those viruses which are the common contaminants of the animal species used.

Millipore membrane filtration was used satisfactorily for the separation of mixtures when the size ranges of 2 viruses were broad, *i.e.*, >100 mμ (SV5) and <50 mμ (SV40). The filtration method obviously was not suitable for separation of measles virus and SV5 (myxovirus) which are in the same size range (11,12).

Summary. Double infection of SV40 and measles virus occurred in single cells of kidney cultures prepared from a green monkey. Nuclei infected with both viruses could be recognized by using acridine orange fluorescence at pH 8.0. Cultures triply infected with SV40, measles, and SV5 viruses did not interfere with poliovirus yield, but poliovirus stocks derived from such cultures were con-

taminated with several viral agents. Therefore, it would be advisable to examine uninoculated cultures for the presence of possible endogenous contaminants.

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Received September 9, 1965. P.S.E.B.M., 1966, v121.

Serum Transaminases and Aldolase in Rats Inoculated with *Trypanosoma lewisi.** (30832)

B. D. NELSON AND D. R. LINCICOME†
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of HEW, Bethesda, Md.

It is usually assumed that parasitic infections have some detrimental effects on the host organism. In the case of the *Trypanosoma lewisi*-rat relationship such effects are not readily apparent. There is, in fact, evidence that the relationship may be mutually satisfactory in that the parasite contributes substances which enhance the growth of the rat(1,2). Although *T. lewisi* augments the growth rate of the host, it seems likely that it would also produce a stress on the host by utilizing host materials for its own growth, elaborating metabolic by-products which affect the host's metabolism; or more simply, by the production of mechanical damage to the host as a result of high numbers of parasites in the tissues or blood stream. Since a large number of non-specific stresses are known to result in the elevation of serum enzymes(3-6), it was felt that any stress imparted to the rat by *T. lewisi* may be manifested by the elevation of serum enzymes. A study, therefore, was initiated to determine the changes in the levels of several rat serum enzymes during the course of *T. lewisi* population growth and decline. In addition, the relative activities of these enzymes were established in trypanosome cells which were isolated from rat blood.

Materials and methods. Two hundred fifteen immature Sprague-Dawley female rats, each weighing approximately 75 g, were employed. Trypanosome infections were initiated by intraperitoneal inoculation of the "L" isolate of *Trypanosoma lewisi*. Cells for inoculation were harvested and prepared as a pure suspension in physiologic saline according to the method of Lincicome and Watkins (7). The inoculum in one series of experiments contained 5×10^7 cells. In a second experiment two levels were employed: 10^2 and 5×10^7 cells. Estimations of all trypanosome populations in peripheral tail blood and in saline suspensions were made with a hemocytometer, using Toisson's fluid as the diluent. All dilutions were at 1:200.

Blood for serum enzyme analysis was drawn by cardiac puncture of rats under sodium pentobarbital anesthesia. Each rat was sampled only once. Samples to be analyzed

* Supported in part by U.S.P.H.S. Research Grant A1-03409.

† Professor of Zoology, Howard Univ., Washington, D. C., on sabbatical leave as Visiting Scientist, Laboratory of Physical Biology, Nat. Inst. of Arthritis and Metab. Dis.