to weaning were fed rations high in carbohydrate, fat, protein, and protein and fat with and without Vit. B_{12}. 2) The greatest degree of deficiency was consistently observed in rats on high protein and high protein-high fat rations. Since Vit. B_{12} increased ability of rats to utilize excess protein, one of the important functions of this vitamin appears to be the metabolism of excess amino acids for non-protein functions. 3) Differences in growth on various diets, however, may be due to degree of intestinal synthesis of Vit. B_{12} and extent of coprophagy.

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Cytopathogenic Effects of Hemadsorption Virus Type I.* (24207)

R. Q. MARSTON†
Medical College of Virgina, Richmond

Several viruses causing human disease induce formation of large multinucleated cells in tissue culture preparations. These include measles(1), mumps(2), croup associated virus(3-4), and 2 recently described myxoviruses. Chanock, *et al.*(5), have termed these latter hemadsorption viruses Types I and II. They were isolated from children with respiratory illnesses and appear distinct from previously described viruses. Attention was called to the fact that hemadsorption virus Type I has been isolated from uninoculated HeLa cell cultures. The unique cytopathogenic changes caused by this virus in HeLa cell cultures have been followed by time-lapse phase-contrast cinemicrography.

Materials and methods. Hemadsorption virus Type I (Mill's strain) was obtained from Dr. Wallace Rowe following its isolation from uninoculated HeLa cultures. The virus pool for these studies was second passage LoHi‡ virus titering approximately 10^5 $T.C.D._{.50}$ per ml. Photographic chambers were constructed by attaching #1 cover slips on each side of perforated stainless steel slides. The top cover slip was placed slightly eccentrically, leaving a small opening at one edge for access to the chamber. This was subsequently sealed with a drop of paraffin. A suspension of small clumps of HeLa cells in Eagle's basal medium, 5% calf serum, 5% cord serum, penicillin 100 units and streptomycin 100 μg/ml, was introduced into the chamber which was then sealed and inverted until cell attachment occurred. These preparations were carried on growth medium until colonies reached optimal size for photography, at which time the growth medium was removed, the cells washed twice with buffered

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† Markle scholar in Medical Sciences.

‡ Designates a stable cell line established from explants of a lymph node from patient with rheumatoid arthritis. The strain grows on glass in an epitheloid pattern with a mean generation time of 24 hours. The viral spectrum is similar to that of HeLa. Tumors have been produced by inoculation of irradiated cortisone treated mice.

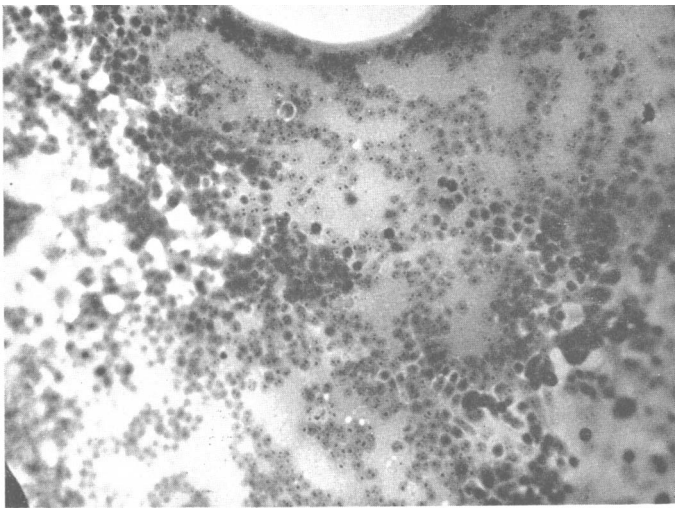

FIG. 1. Cytopathogenic effect of hemadsorption virus Type I 36 hr after infection. Hematoxylin and eosin. × 160.

salt solution, and the chamber inoculated with 0.1 ml of virus in 0.4 ml medium consisting of 78% Medium 199, 20% trytose phosphate, 2% calf serum, penicillin 100 units and streptomycin 100 μg/ml. Cytopathogenic changes were documented through an incubated phase-contrast microscope by intermittent photography at a speed of 4 frames minute. Stained cultures were prepared by the colloidian membrane technic(1).

Results. Detectable changes occurred approximately 12 hours after inoculation although the time of initiation of changes in different preparations and in different colonies in the same chamber was variable. Early changes consisted of appearance of multinucleated cells which, through a process of coalescence of cytoplasm of adjacent cells, increased rapidly in size until hundreds of nuclei were included in a single cell (Fig. 1). An example of fusion of 2 cells is shown in Fig. 2. After a few hours the central area of many lesions detached from the glass and fell away into the medium leaving, characteristically, a hole lined by a syncytial-like layer of cells (Fig. 3). During 12 to approxi-

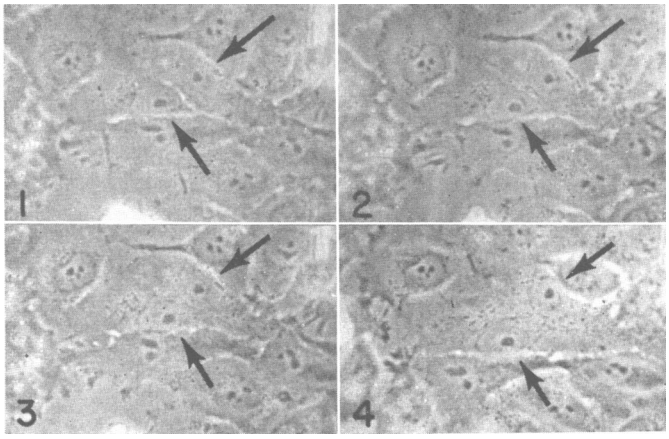

FIG. 2. Excerpts from motion picture record of HeLa cell fusion. Ten min. interval between frames. Frame 1—Arrows point to nuclei of 2 separate cells. Frame 2—Fusion has started at lower right border. Frame 3—Complete fusion. Frame 4—Nuclei have drifted apart. Living image. × 320.

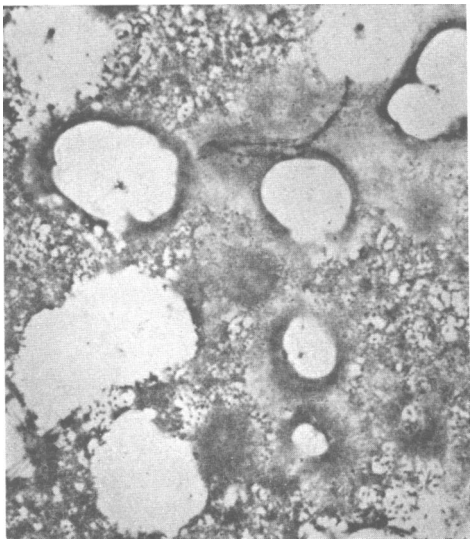

FIG. 3. HeLa culture 48 hr after infection with hemadsorption virus Type I. Hematoxylin and eosin. × 60.

mately 36 hours after infection when the marked cytoplasmic membrane changes were occurring, there was striking lack of morphological change in other organellae of the cells. The nuclei maintained their integrity and motility, including the rotary motion frequently seen in HeLa cells. Mitochondria similarly continued to exhibit essentially normal motility and morphology. Some decrease in nuclear size did occur late in the infection but by this time, distortion of the whole culture made further evaluation difficult or impossible.

We have not observed nuclear division in the absence of cytoplasmic division, although under low power several hundred nuclei in a single cell can be followed for many hours. It is quite possible that this process may occur, but our studies clearly rule it out as a major process in the development of the large multinucleated cells characteristic of infection with this virus. Mitoses within the developing multinucleated cells similarly have not been observed, but normal mitoses immediately adjacent to the developing lesion have been photographed and have been seen frequently in stained preparations.

Since the virus which we used had been isolated from presumably uninoculated HeLa cultures, and since both giant cells and multinucleated cells are seen in stock HeLa cultures, the possibility that these represented a limited viral effect was considered. However, on several occasions we have apparently mistaken such cells for early viral lesions and photographed them for hours with no evidence of progression, although progressive changes were occurring in other areas of the culture.

Discussion. Enders(6) noted that after inoculation of tissue culture preparations with measles virus, the appearance of multinucleated cells precedes by many days the production of inclusion bodies or other changes, and postulated that multinuclearity in this instance is due to coalescence of the cytoplasm of adjacent cells. Chanock(3) has described changes in croup associated virus inoculated cells quite similar to those described here. Henle *et al.*(2) have shown that cell fusion is the process responsible for multinuclearity in mumps infected cultures and postulates a relation between hemolytic and "cytolytic" properties of mumps virus. Hemadsorption virus Type I may cause hemolysis of red cells, but whether the remarkable effects on HeLa cell membranes is due to this property or to impairment of cellular mechanisms responsible for maintainance of membrane integrity is unknown.

Summary. Cytopathogenic effects of hemadsorption virus Type I in HeLa cells as studied by time-lapse phase-contrast cinemicrography and by colloidian membrane preparations have been described. The cytopathic changes are characterized by formation of large multinucleated cells followed by focal loss of cells leaving multiple holes in the cell sheet. Coalescence of the cytoplasm of adjacent cells precedes marked alteration of intracellular organellae by many hours.

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Studies on Chloride of Bone in Cat and Rat.* (24208)

CHARLES C. LOBECK† (Introduced by Gilbert B. Forbes)
Dept. of Pediatrics, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

The chloride space, calculated from values for bone chloride content, is frequently used as a measure of extracellular fluid volume of bone. Use of this calculation rests on the assumption that chloride is not located in cells or on crystals but solely in extracellular fluid. However, it has been observed by Nichols and Nichols(1) in dogs and by ourselves in rats, that the chloride space is often greater than one would expect if total bone water content was extracellular fluid. This discrepancy is complicated by the difficulties of chloride analysis in a tissue that contains small amounts of chloride with the possibility of associated errors due to blood content of cortical bone or contamination of bone samples with marrow and blood.

The purpose of this paper is to present the results, in cats and rats, of an accurate method of chloride analysis for cortical bone. Use was made of Br^{82} to determine exchangeability of the chloride and as a check on the accuracy of chloride analysis. Cr^{51} labeled red cells were also used to determine contributions made by blood content of bone to its water and chloride content.

Procedure. Cats of varying weights but unknown ages were anesthetized with Dial with Urethane (Ciba) injected intra-abdominally. Venesections were made in both saphenous veins. A solution of KBr^{82}‡ in normal saline and a suspension of Cr^{51} labeled red cells were injected into one saphenous vein by the method of Barnett and Fellers(2), using a maximum of 8 ml of normal saline as wash solution. The other venesection was used solely for withdrawal of samples. Previously an aliquot of the KBr^{82} solution had been removed for counting as a standard, leaving 10 μc to be injected. The Cr^{51} labeled cell suspension was prepared by withdrawing 3 ml of blood and incubating it for 30 minutes at room temperature with approximately 100 μc of Cr^{51} in the form of $Na_2Cr_2O_7$.§ Red cells were washed 3 times with normal saline, reconstituted to original volume, an aliquot removed for counting as a standard and the remaining cell suspension injected into the cat. Two hours were allowed for equilibration of both isotopes, since previously determined disappearance curves of both KBr^{82} and Cr^{51} labeled red cells, injected intravenously, reached a constant slope by 2 hours after injection. At the end of this period, blood was withdrawn for counting and analysis. At the same time the blood supply of left upper extremity was occluded with a tourniquet and the limb amputated at elbow. The radius and ulna were dissected free, the periosteum removed by scraping and the marrow blown out with compressed air. The bone was then broken into small pieces, cleaned of remaining marrow by blotting with filter paper, placed in a tared vial and weighed immediately. For determination of chloride content of rat bone, groups of Holtzman rats of known ages were exsanguinated after ether anesthesia. All long bones of young rats were removed but, in older animals, only the right upper and lower extremities were taken. Before weighing in tared bottles, the bones were cleaned using the same procedure as for cat bone.

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† Present address: Univ. of Wisconsin Med. School, Madison.

‡ Oak Ridge National Lab.

§ Rachromate, Abbott.