tems of cells maintain concentration gradients of Na and K ions under these conditions at normal level even though rate of K exchange in the denervated muscle is somewhat decreased(10).

Evaluation of total absolute ion changes makes it possible to estimate changes in extracellular fluid and the intracellular muscle phase. The results indicate that following denervation, only the intracellular muscle phase decreases, while extracellular space remains unchanged even when muscle atrophy reaches 50%. Conclusions in earlier reports concerning increase of Na and Cl ions in the denervated muscle(1,2,3,7,8) must be modified in view of our evidence that the increase of Na and Cl ions/unit weight of denervated muscle is only relative, due to volume of extracellular fluid remaining unchanged while volume of intracellular phase decreases.

Summary. The content of potassium expressed in relation to non-collagenous protein nitrogen is normal even 20 days after denervation in muscles of the rat. Following denervation only the intracellular muscle phase decreases. The drop in K content (K/muscle) and FFDS (weight/muscle) reaches 50%. At this time the extracellular space (Na/muscle, Cl/muscle) remains unchanged. Ion composition and dry muscle weight gradually return to normal after reinnervation and restitution of motor function.

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Histological Changes in Cells Infected with Columbia SK Virus. (25694)

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Histological evidences of Columbia SK virus infection have been of interest because the nature of pathological changes and site of multiplication of the virus have not been completely identified, and also because we hoped to identify the step at which virus propagation was interrupted by the antiviral substance "Propionin"(1). Some clarification of the first of these problems is given in this paper, in which histological appearance of infected cancer cells is described.

Procedure. After serial passage in Ehrlich ascites tumor cells, grown intraperitoneally in young white mice, an adapted strain of Columbia SK virus was obtained which produced severe oncolysis(2). Such infected ascites fluid, aspirated with 2 ml of saline from abdominal cavity before mice died at 3 to 5 days after virus inoculation, contained about 10^6 LD_{50}/ml of virus. The supernatant of this fluid, which contained the same titer of virus, was diluted 10^{-3}, and 0.1 ml mixed with 0.3 ml of fresh, uninfected Ehrlich ascites, drawn 5 to 10 days after implantation, and inoculated intraperitoneally into new mice. Three or 4 days later cells were withdrawn for morphological study, and stained as follows: *Giemsa*—Smears were fixed with methanol and stained by usual Giemsa solution. *Lipoid*—Smears were fixed with formalin and stained with Sudan III, or Sudan Black B, saturated solutions in 70% alcohol, then counterstained with Giemsa solution. *Tetrazolium*—Immediately after aspiration a drop of ascitic fluid was mixed on a slide with a drop of 2% 2,3,5-triphenyl-2H-tetrazolium

chloride solution in saline. A cover slip was applied, ringed with petrolatum, and the slide examined after 30 minutes at room temperature. *Acridine orange*—Smears were fixed with formalin and stained with 0.1% acridine orange R at pH 3.0, adjusted with HCl by the method of Armstrong(3), for 30 minutes, then washed with water and dried. *Fluorescent antibody*. The indirect method of Weller and Coons(4) was applied, using immune rabbit serum against Columbia SK as previously described(2), including 2 adsorptions of nonspecific substances by washed Ehrlich ascites cells, and producing an anti-hemagglutinin titer of 1:640. Fluorescein conjugated sheep anti-rabbit globulin was obtained commercially. Smears were fixed with acetone for 10 minutes, then exposed 30 minutes to 1:10 dilution of anti-SK rabbit serum diluted with phosphate buffered saline. After washing gently with buffered saline, they were overlaid for 30 minutes with a 1:10 dilution of fluorescein-conjugated sheep globulin. After final washing, smears were mounted in buffered glycerin and examined for presence of intracellular antigen under fluorescence microscope with a dark field condenser. Normal rabbit serum replaced anti-SK rabbit serum in controls.

Results. Characteristic appearances of normal and infected tumor cells are shown in Fig. 1. Progress of the infection as stained by Giemsa solution is shown in 1, 2 and 3. No. 1 shows uninfected or control tumor cells; No. 2, 2 days after infection, shows extensive vacuolization and loculation of the cytoplasm, in which many vacuoles are larger than those of control cells; No. 3, 4 days after infection, indicates massive oncolysis present in late stages of infection, disintegration of cytoplasm, and shrinking and distortion of the nuclei. Nos. 4, 5 and 6 show the same stages stained by Sudan Black B. A number of stained vacuoles are seen in control cells of No. 4 but these appear not to be the same vacuoles as the smaller type seen by Giemsa staining; No. 5, 2 days after infection shows a moderate increase in Sudan Black B stained vacuoles, indicating an increase in lipoid; terminal stages, in No. 6, show the still greater accumulation present in some cells, but virtual absence in others, highly loculated, which may indicate loss of fat before disintegration. No. 9, by contrast, stained with Sudan III, in a cell 4 days after infection, shows presence of neutral fat in the large terminal locules. The difference in staining with the 2 fat stains is definite but not explained. Normal cells show little or no staining with Sudan III. Nos. 7 and 8 show normal tumor cells stained with triphenyltetrazolium chloride, and infected cells at 2 days; the brilliant red color produced by reduction of the dye in vacuoles of controls is entirely absent in infected cells. No. 10 shows uninfected cells stained with acridine orange, as seen with fluorescent microscope; nuclei are green and cytoplasm brilliant orange. After 2 days, in No. 11, the usual intense vacuolization is seen, vacuoles being unstained, and some cells have lost most of their orange coloration; after 4 days, in No. 12, green ghosts may be seen and only a single normal, or recently infected, cell with orange cytoplasm. Finally, Nos. 13, 14 and 15 show increase of fluorescent antibody as the disease progresses 2, 3, and 4 days after infection. Initial fluorescence is peripheral, in small areas, but this evidence of virus antigen progresses steadily until nearly the whole cell may be involved.

These results indicate that infection produces: a. vacuolization and loculization (Giemsa), b. increase of lipoid vacuoles (Sudan Black B and Sudan III), c. failure of reduction of tetrazolium (tetrazolium), d. disappearance of cytoplasmic ribonucleic acid (acridine orange), and e. gradual increase in virus antigen, probably starting in the cytoplasm (fluorescent antibody).

Discussion. Two processes go on simultaneously in the infected cells described, *i.e.*, destruction of the cell and propagation of the virus. Positive evidence of virus propagation was afforded only by the fluorescent antibody technic as other methods of staining showed what more probably were manifestations of damage to the cells. If the Columbia SK virus is an RNA virus, like the polio virus which it resembles in some respects, the disappearance of ribonucleic acid stainable by acridine orange is unexpected and might be interpreted as the result of replacement of normal RNA

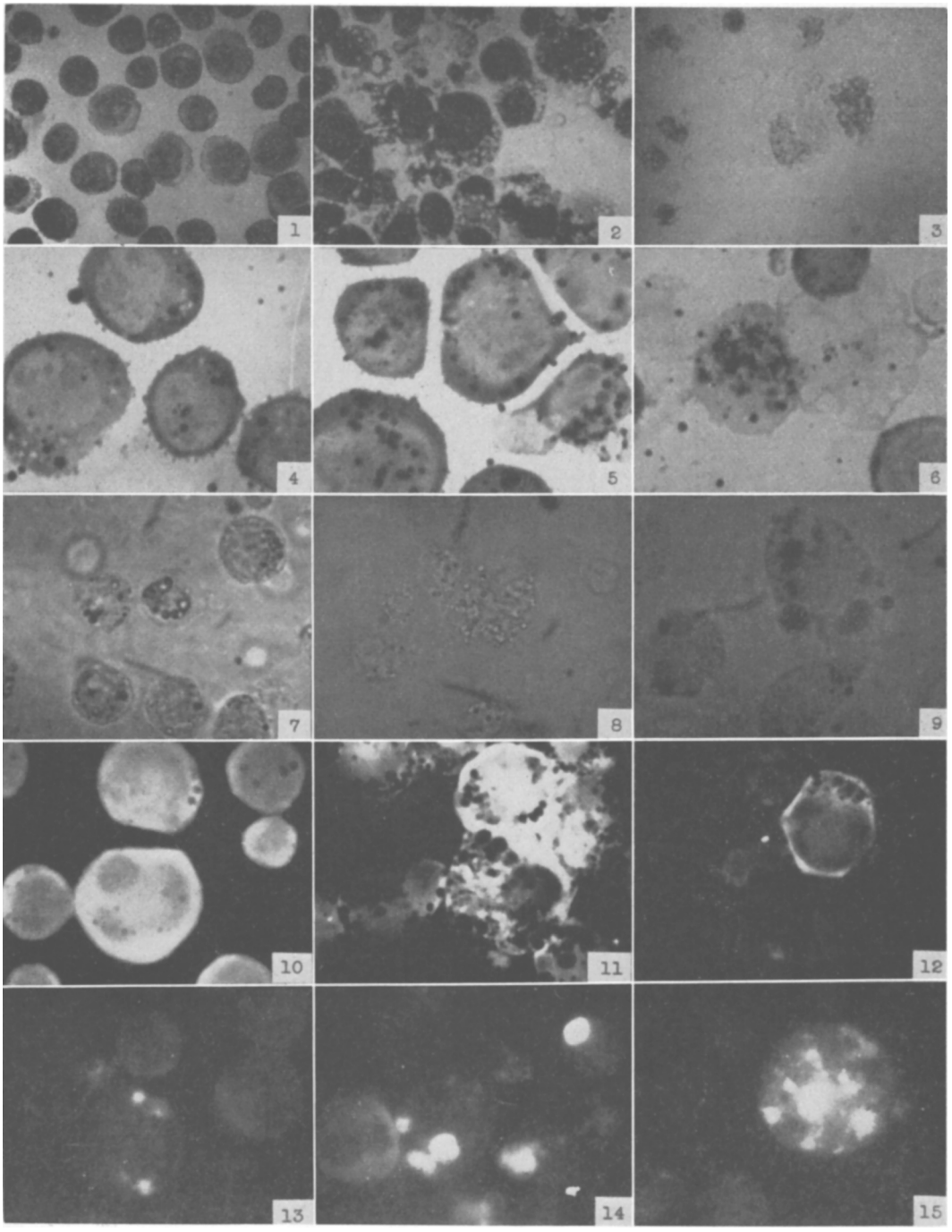

FIG. 1. Ehrlich ascites cells showing changes produced by Columbia SK virus infection (see text for details).

of cytoplasm by virus in which RNA was occluded by a protein matrix or the protein viral coat. However, Niven(5) has shown that acridine orange can penetrate through protein to stain virus RNA in some cases. The changes of lipoid and tetrazolium staining indicate enzymatic aberrations, probably part of the oncolytic process, which might be sub-

ject to further analysis by enzymatic and metabolic studies. It is hoped that the histological changes described will clarify the point of action of chemotherapeutic agents.

Conclusions. The changes produced in Ehrlich ascites tumor cells by Columbia SK virus are described, using various staining technics including fluorescent antibody staining.

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Protamine and Conversion of Mevalonic Acid to Cholesterol by Liver Homogenates.* (25695)

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Preincubation of essentially whole homogenates of rat liver with crystalline ribonuclease (RNAse) abolishes capacity of these homogenates to convert mevalonic acid (MVA) into non-saponifiable material (NSF) or cholesterol (CHL)(1). Evidence has been presented that RNAse acting in association with easily sedimentable constituent of homogenate (2,3) destroys a system concerned with maintenance of ATP(4,5,6). This system appears to require a polynucleotide, presumably "free" or "combined" RNA as an essential component since inactivation may be prevented or reversed by a "nucleoprotein" of autoclaved liver extract, or by relatively large amounts of RNA, or by DNA, or by a number of "unnatural" anionic polymers including polyethylene sulfonate (PES) or heparin(7,8). It has now been found that capacity of the system for maintaining adequate ATP levels as judged indirectly by utilization of MVA for biosynthesis of NSF is lost by preincubation of the homogenate with protamine. This protamine inhibition is associated also with absence of ATP in the homogenates and is, furthermore, preventable with RNA, DNA, or PES and, using PES as an example, is associated with a level of ATP in the homogenate equal to that of control. Reasons are presented here for believing that preincubation of liver homogenates with protamine results in formation of a protamine-RNA complex that renders RNA unavailable for its role in maintenance of ATP levels adequate for phosphorylation of MVA.

Methods and materials. The biosynthetic experiments involved preincubation of 5 ml aliquots of a 200 x g supernatant fraction of rat liver homogenate prepared as previously described(1-9) with 5-8 ml amounts of solution containing 1 mg ATP, 10 mg DPN, 100 mg sodium succinate, various amounts of protamine (neutralized), and other supplements to be studied. Each flask was aerated with a stream of oxygen and preincubated with agitation for 30 min at 37°. Following preincubation each flask was opened and 1 ml MVA-2-C^{14} solution added, the contents were aerated and the flasks then reincubated for additional 3 hrs. After final incubation, homogenates were saponified, extracted with petroleum ether, the ether extracts dried with sodium sulfate, filtered, evaporated, taken up in scintillation mixture and counted. Previous studies have shown(1,9) that under experimental conditions employed the counts found in the NSF fraction are essentially CHL or other digitonin-precipitable material. In experiments involving determination of ATP

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