strated the presence of a rapidly migrating component (V) and of a material with a mobility between β- and γ-globulin (τ) in each of 10 cerebrospinal fluids studied with the paper-electrophoresis technic. The rapidly migrating component, designated as X-protein in this laboratory, has been previously described(1) and has been found in each fluid studied in the present investigation. The τ component was noted in less than half of the fluids analyzed. This disagreement with the results of the German workers may possibly be due to the types of patients studied or to the difference in the procedures used for concentrating the cerebrospinal fluid.

Summary. 1. The results of a detailed investigation of the distribution of two rapidly migrating components and of other protein components of cerebrospinal fluids have been presented. A component (X-protein) migrating about 20% faster than the albumin could be demonstrated in every fluid by electrophoresis in phosphate at pH 7.85 and ionic strength 0.05. In addition a component (X_1) migrating 8-14% faster than the X-protein was observed in 20% of the cases. 2. Analyses of 49 patterns of the most frequently encountered type and 16 atypical patterns are presented. No individual protein component was responsible for the higher protein concentration observed in some of the fluids. A possible relationship between the X_1 component and the occurrence of convulsion in patients was indicated.

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Simultaneous Multiplication of Two Different Rickettsiae in the Same Cell. (19967)

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The observations of many investigators have amply shown that infection of a host (bacterial, plant, or animal) by one virus may, under certain conditions, afford temporary protection against infection by another, *i.e.*, interference phenomenon. However, interference is not the universal result of duplicate infections, since many instances of dual virus infections have been described(1,2). Several investigators have reported that two different animal viruses can multiply simultaneously in the same cell. By using viruses that form recognizable intracellular inclusions, studies(3,6) were made to demonstrate cytological evidence that individual cells may be invaded by, and become host to, two different viruses. Levaditi and Schoen(4) observed simultaneous appearance of Negri and Guarnieri bodies in the epithelial cells of rabbit cornea. Syverton and Berry(5) showed that cells of the Shope rabbit papilloma can be superinfected by extraneous viruses. McWhorter and Price(7) demonstrated the presence of inclusions of both tobacco mosaic and tobacco etch viruses in the same cells of doubly infected tobacco plants, and concluded that their presence together indicates simultaneous growth of two plant viruses in the

same cell. Simultaneous multiplication of two bacterial viruses in the same bacterium was also demonstrated(8-10). Pneumonia virus of mice and influenza viruses, or mumps and influenza viruses, have been propagated simultaneously in the respiratory tract of mice and in the allantoic cavity of chick embryos, respectively(11). It has been reported(12) that both influenza A and B viruses have been carried simultaneously through consecutive passages in the chick embryo. Takemori(13) observed in mixed tissue culture that the virus of lymphogranuloma venereum and murine typhus rickettsiae grow simultaneously in the same cell.

The obligatory intracellular parasitic nature of rickettsiae makes the demonstration of dual rickettsial infections in single cells of some theoretical interest. Since rickettsiae can easily be seen microscopically in stained preparations, dual rickettsial infection of the same cell can readily be recognized. Experiments reported here demonstrate the simultaneous multiplication of both *Rickettsia tsutsugamushi* and *Rickettsia typhi* in the same cell.

Materials and methods. The Wilmington strain of murine typhus (*R. typhi*) and the KMT strain* of scrub typhus (*R. tsutsugamushi*) were employed in the experiments. A 10% suspension in buffered saline of mouse livers and spleens infected with *R. tsutsugamushi* was mixed with equal volume of a 10^{-3} dilution of chick embryo yolk sac infected with *R. typhi* and 0.2 ml amounts of this mixture were injected intraperitoneally into 5 mice. At 7 to 10 days after infection when the signs of disease developed, the mice were sacrificed, a 10% suspension of infected livers and spleens was prepared in buffered saline and passed intraperitoneally to 5 new mice as above. Thus far 5 serial passages have been made. Since the two rickettsiae are known to multiply similarly in the cells lining the peritoneal cavity, smears were prepared from the surface of the spleen or the peritoneum, and stained with Giemsa.

Results. Microscopical examinations of the stained smears from each passage revealed, besides cells containing either *R. tsutsugamushi* or *R. typhi*, cells showing simultaneous multiplication of both *R. tsutsugamushi* and *R. typhi* in the cytoplasm.

Some of such doubly infected cells are shown in Fig. 1 A-F. The respective rickettsiae in the same cells can easily be identified, being different in size, form, staining properties and location. *R. tsutsugamushi* is thicker than *R. typhi*, ellipsoidal, often appearing as a diplococcus, colored deep purplish with Giemsa stain, and usually located near the nucleus. It must be emphasized that apparently the two rickettsiae grow quite independently in the same cytoplasm, each forming respective "colonies." Although the two rickettsiae have been carried simultaneously through 5 consecutive passages in mice, apparently without interfering with the growth of each other, maximal growth of *R. typhi* in some of the doubly infected cells seemed to reduce the normal multiplication of *R. tsutsugamushi* in the same cytoplasm. Takemori (13) observed simultaneous growth of the virus of lymphogranuloma venereum and *R. typhi* in the same cell of mouse embryonic tissue in mixed tissue culture. As the microphotographs were not taken at that time, two such doubly infected cells, in schematic form, are also shown in Fig. 1 G for the sake of comparison.

Discussion. This is the first time that dual infections of individual cells with two different obligatory intracellular parasitic agents have been observed microscopically with absolute certainty. In this connection it is of interest to note here that double and multiple infections of single cells with one virus were often reported in the case of the viruses of the psittacosis-lymphogranuloma group(14) and the possibility must be considered that similar double and multiple infections of cells may occur with either one or two animal viruses of other groups. With the use of the electron microscope(15) attempts were made to analyze the changes produced in cells by various viruses. Particularly interesting is a composite picture of a cell(15) which shows but a portion of the cell completely destroyed and disintegrating with virus lying free. The intact portion of the cell still preserves the nor-

* Isolated by Prof. N. Ogata in 1951 in Akita Prefecture.

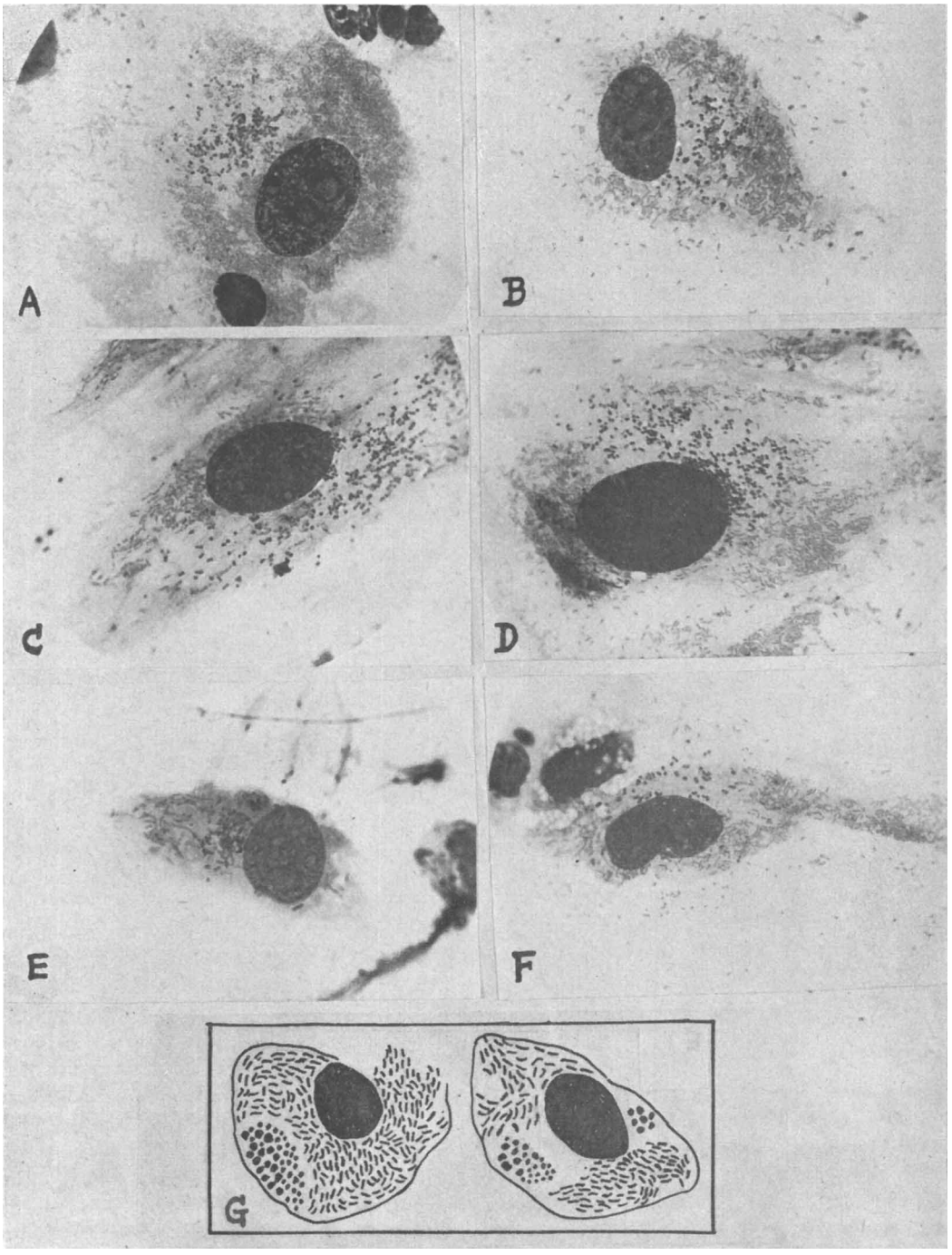

FIG. 1. A-F. Microphotographs by S. Noda. Simultaneous growth of *Rickettsia tsutsugamushi* and *Rickettsia typhi* in the same cell. Smear, stained with Giemsa. G. Simultaneous multiplication of *Rickettsia typhi* and the virus of lymphogranuloma venereum in the same cell (in schematic form). 1000 ×.

mal detailed fibrous microstructure and it seems reasonable to assume that this portion of the cell may become host to a second virus simultaneously inoculated.

The results obtained in these experiments suggest a probable explanation of the mechanism underlying the simultaneous consecutive passages of two animal viruses reported by previous investigators(11,12).

Summary. When mice were inoculated intraperitoneally with a mixture of *R. tsutsugamushi* and *R. typhi* it was found that both rickettsiae multiply simultaneously in the cytoplasm of the same cells lining the peritoneal cavity. The significance of this finding with regard to the simultaneous growth of other viruses in the same cell is described.

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Suppression of Gastric Acidity by Radio Krypton.* (19968)

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Radioactive Krypton, when contained in a balloon inflated in a dog's stomach, provides a safe, convenient procedure for suppressing gastric acidity. The induction of achlorhydria by roentgen therapy has been discarded because of inability to achieve adequate, intense selective radiation: the liver may suffer damage and necrosis, and in a few instances fatality has resulted(1,2). Simon(3) reported the use of P^{32} in suppressing acidity; while effective, the method was technically difficult because it involved the use of a double balloon with the phosphorus absorbed into cotton cemented to the outside of the inner balloon. McKendry(4) and Forse, McKendry and Webster(5) have used I^{131} for the same purpose, instilling it in solution so that it formed a thin film between the walls of a double walled balloon. In a series of well controlled experiments they were able to decrease the volume of secretion by gastric pouches without significant alteration of pH. *Exact local radiation* can be given only by an isotope with beta and no gamma emission. Most beta emitters such as P^{32} cannot be used inside a balloon because they must be dissolved in water—the water absorbs more than 90% of the radiation from the isotope in balloons of adequate size. However the absorption of beta radiation in a gas-filled balloon is negligible.

Two preliminary survey experiments were done with Argon 41 because of its 1.2 Mev beta emission. The dosage unit used is the "roentgen equivalent physical" or "rep" defined as that amount of beta radiation which, under equilibrium conditions, releases in one gram of air as much energy as one roentgen of gamma rays. A beta dosage of about

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