in weanlings of a diverse mouse strain on combination with ascites tumor.

Summary. Abnormal response to ascites tumor on passage in weanlings from a conventionally maintained colony of Swiss mice indicated a high rate of infection with murine hepatitis virus (MHV). It was not encountered, however, during weekly transfer of the tumor over a 2-year period in weanlings from a specific pathogen-free colony of the same Swiss line. Two strains of the virus, MHV(SR)1 and 3, were differentiated by their behavior in Swiss and Princeton (Pr) mice. The host specificity of strain 1 was altered by passage in one-day old Pr nurslings and in presence of the tumor but that of strain 3 was unaffected.

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Autoradiography with the Agents of Psittacosis and Trachoma.* (28529)

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Microorganisms associated with psittacosis, lymphogranuloma venereum, and trachoma have been conveniently and perhaps appropriately designated as PLT agents(1). However, phylogeny remains an issue(2). Group associations are based on morphological(3,4), serological(5), and biochemical(6) criteria. In addition comparison of developmental cycles by fluorescence microscopy of acridine orange-stained preparations showed unique cytochemical patterns(7) which were not observed with *Rickettsia burnetti,* vaccinia, or adenovirus. Recently, Pelc and Crocker(8) noted a "low utilization" of thymidine by an unidentified strain of psittacosis. Pollard and Starr(9) reported that thymidine did not reverse the inhibitory effect of aminopterin on the TT strain of psittacosis in contrast to its reversal effect on the host cell. It was stated (10) on the basis of inhibition studies that the psittacosis agent cannot utilize preformed thymidine. Bernkopf *et al.*(11) reported incorporation of tritiated thymidine by the T'ang strain of trachoma during a late stage of development. Thymidine utilization by the agents of psittacosis and of trachoma has been reexamined by autoradiographic technics. Both agents were tested under similar experimental conditions.

Methods and materials. The McCoy cell line(12), derived from human synovial tissue, was grown on coverslips in Leighton tubes. Medium consisted of mixture 199 (Microbiological Associates) plus 10% heat-inactivated calf serum, 0.5% lactalbumin hydrolysate, and 0.1 mg streptomycin per ml. Psittacosis agent (TT strain)(13) was propagated in chorioallantoic fluid of 7-day old embryonated chicken eggs, pooled, ampouled, and stored at –40°. Trachoma agent (T'ang strain)(14) was adapted to McCoy cells(15). Stock was obtained from pooled sonicates of infected cells, ampouled, and stored at –40°.

Stocks were titrated by a modification(16) of the infected cell count technic described by Weiss and Huang(17). Inocula were prepared by dilution of stock in nutrient medium to insure infection of 60 to 80% of the cells. Both agents were treated alike in parallel experiments. In one series of tests, McCoy cultures were inoculated and after a 2-hour absorption period at 37° medium

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was decanted. Cell sheets were washed in nutrient medium. At this time, nutrient medium supplemented with tritium-labelled thymidine was added. At specified intervals for 72 hours thereafter, medium was decanted from triplicate tubes and cold medium was added for 30 minutes to remove excess label. Autoradiographs were prepared. In another series of tests, tritriated thymidine was added for a 1-hour period at intervals after infection. Cells were washed in cold medium for 30 minutes and autoradiographs were prepared.

The methyl-labelled thymidine had a specific activity of 6.7 c/mmole. It was used at a final concentration of 0.1-0.5 μc/ml medium. Coverslip preparations were fixed in acid-alcohol for 10 minutes and either stained with acridine orange(7) or mounted cell-side-up on glass slides with Permount. After drying overnight in a dark-room, preparations were dipped into Kodak Nuclear Track Emulsion NTB 2 which was prewarmed to 45°. Slides were conveniently placed into neoprene-covered test tube racks, dried in front of a fan, and put into a light-proof box for 4 days. After development and fixation by routine dark-room procedures, some preparations were counterstained with Giemsa.

Results. Acridine orange-stained preparations were used as a guide to the maturation stage of the agents(7). Under the experimental conditions cited, no significant incorporation of thymidine by either the agent of psittacosis or of trachoma was noted during any stage of their developmental cycle (Fig. 1).

In all experiments, label was incorporated into nuclear material as would be expected.

Discussion. Our results are in agreement with those of Pelc and Crocker(8) who noted a "low utilization" of thymidine by an unidentified strain of psittacosis grown in Hela cells. They stated that the base ratios of the psittacosis agent were within normal limits and that the low level of thymidine incorporation was not due to lack of thymine in the DNA. Thymine was found in the DNA of psittacosis strain 6BC by Ross and Gogolak(18). To account for the presence of thymine in the DNA, it was proposed(8) that

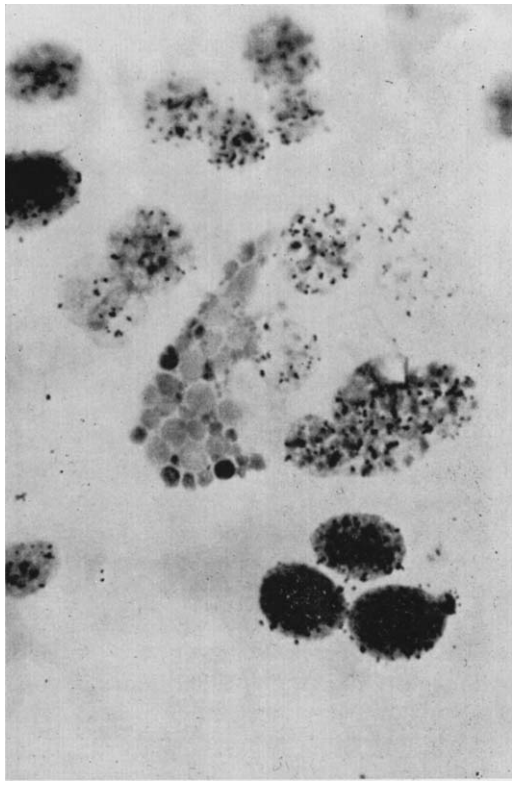

FIG. 1. McCoy cell line infected with the TT strain of psittacosis, showing large grape-like cluster which fluoresces shades of red, orange, yellow, and green when stained with acridine orange(7). Only the nuclei of the cells are labelled. Approximate magnification 850×.

thymidilic acid was synthesized from uridilic acid by mechanisms and pathways suggested by Humphreys and Greenberg(19) for rat thymus. Thymidine does not appear in this scheme. Our results differ from those of Bernkopf *et al.*(11) who noted incorporation of tritiated thymidine only during the late stages (40-48 hours) of development of the T'ang strain of trachoma in FL cells. Their observations indicated that DNA synthesis is most active in later stages, when many particles acquire infectivity, while RNA synthesis occurs at an earlier stage. In previous studies with the TT strain of psittacosis(7), it was shown by cytochemical staining procedures that DNA synthesis occurs throughout the developmental cycle. Thus, removal of the RNA-staining (red) matrix of the early inclusion with ribonuclease unmasked a DNA-staining (green) inclusion. This stage

was further characterized as noninfective, nonantigenic for fluorescein-tagged antibody, and Feulgen positive. Furthermore, thymidine did not reverse the inhibitory effect of aminopterin on the maturation of the TT agent in McCoy cells(9) but did exert a reversal effect on aminopterin-inhibited adenovirus type 12(20). In view of these combined observations, it may be argued that significant synthesis of DNA occurs early in the developmental cycle. Therefore, we would expect to find incorporation of thymidine during all stages of the growth cycle if it occurred at all. Under the conditions of this study, no significant incorporation of thymidine was noted by either the agent of psittacosis or trachoma.

Summary. Significant incorporation of tritiated thymidine into cytoplasmic inclusion bodies of either the TT strain of psittacosis or the T'ang strain of trachoma could not be demonstrated by autoradiographic technics during any stage of their developmental cycles in the McCoy cell line.

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Viable Tumor Cells in Blood and Lungs of Mice with Metastasizing and Non-Metastasizing Transplantable Tumors. (28530)

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Blood-borne metastasis requires at least 2 separate processes: (a) release of a single cell or group of cells into the blood, *i.e.*, embolism, and (b) establishment and proliferation of emboli in a distant organ, *i.e.*, colonization(1). An embolus does not necessarily mean that metastasis will occur(2), since tumor emboli are frequently found in necropsies of cancer patients lacking visible metastases(3). Furthermore, examination of systemic blood has not shown any direct correlation between embolism and colonization(4,5,6,7). Finally, tumors metastasize preferentially to certain sites(8) that may receive relatively few emboli, like metastases in the adrenal in cases of lung cancer(9). Such considerations indicate that the frequency with which metastases develop is not