reflect the inherent nature of certain viruses to induce proliferative cellular response. Cellular necrosis observed in most of lung nodules is compatible with the fact that polyoma virus destroys cells grown in tissue culture.

Summary. Polyoma virus given intranasally produced primary lung tumors in hamsters. Some animals showed visceral involvement. Growths were largely fibrosarcomatous. Hamsters injected by other routes showed almost no lung neoplasia.

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Received April 11, 1960. P.S.E.B.M., 1960, v104.

Propagation of Trachoma Virus in Cultures of Human Tissues.* (25786)

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Although the etiological agent of trachoma was first described in 1907(1), technical limitations prevented a rapid evolution of the problem. T'ang *et al.*(2) reported on its physical characteristics and propagation in the yolk sac of chicken embryos. By omitting penicillin from the inoculum(2), virus isolation was confirmed by other workers(3-7). Subsequently, Gordon *et al.*(8) reported propagation of trachoma virus in tissue cultures of entodermal cells of chick embryos. This report describes adaptation and serial propagation of trachoma virus in tissue cultures of human origin.

Methods. The strain of trachoma virus (SA-2) was kindly provided by Dr. John C. Snyder, Harvard University(4). It had been passaged 7 times in yolk sacs of embryonated eggs. The virus was propagated by us in yolk sacs of embryonated eggs derived from a PPLO-free flock of chickens§ which were on an antibiotic-free diet. Infected yolk sacs were pooled, homogenized in a Ten Broeck grinder, and diluted 1:10 with PG medium (9). After centrifugation at 3000 rpm for 20 minutes, the aqueous supernatant, containing the virus, was stored in ampoules at dry ice temperature. Human amnion (FAM)(10) and human synovial (McCoy)(11 and personal communication) cell lines were propagated on cover slips in Leighton tubes. Tissue explants from 9-day-old chicken embryos were also used. Nutrient medium was made to volume with Mixture 199 and contained 5% heat-inactivated calf serum, 0.5% lactalbumin hydrolysate, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. When confluent cell-sheets had formed and tissue explant growth was suitable, cultures were washed twice with Mixture 199 and 0.1 ml of the virus inoculum was added to each tube. Nutrient medium without antibiotics was then added to each tube (0.9 ml) and cultures were incubated at 37°C. Culture fluids were changed at 4-day intervals. Duplicate coverslip preparations were treated with Wright's plus Giemsa stain and with acridine orange dye. The latter preparations were examined by fluorescence microscopy(12). Other cultures were frozen and thawed twice, pooled, and inoculated (0.25 ml) into the yolk sac of

* Supported in part by Hartz Mountain Fund and James W. McLaughlin Fellowship Fund.

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7-day embryonated eggs. At 6-8 days after inoculation or upon death of the embryo, yolk sac smears were examined for elementary bodies. Trachoma virus was passaged at 7-day intervals through 5 series of tissue cultures. Successive inoculums to tubes containing fresh tissues consisted of 0.3 ml of a pool from the previous culture. The virus was permitted to adsorb for 2 hours, then nutrient fluid, less antibiotics, was added to each tube (1.5 ml).

Results. The observations of Gordon *et al.* (8) on propagation of trachoma virus in tissues of chicken embryo explants were confirmed. Cytoplasmic inclusion bodies appeared in FAM cells within 72 hours. They occupied a vacuole and stained blue with Wright's plus Giemsa stain. Frequency of infected cells was low and maturation of the inclusion to the distinct elementary body stage was not observed. With acridine orange, the inclusion body was red-orange (RNA-staining) which coincided with the early stages of developing psittacosis virus inclusions as reported by Starr *et al.*(12).

In McCoy cells, cytoplasmic inclusions appeared within 48 hours. They were dense basophilic masses which occasionally occupied a vacuole. They increased in number and in size with age. With successive passage of the agent, inclusions became more numerous (Fig. 1). Thus, approximately 5% and 50% of cells were infected after first and the fifth passage, respectively. In acridine orange-stained preparations from fifth passage cultures, inclusions were red-orange (RNA-staining) (Fig. 2) and later matured to yellow-green (DNA-staining). Multiple stages of maturation indicated that cellular reinfection had occurred. The inclusion body was the only lesion noted.

All of the embryonated chicken eggs inoculated with tissue culture pools developed elementary bodies in the yolk sac. Virus was not infective for weanling Swiss mice inoculated *via* the intracerebral route.

Discussion. Earlier studies indicated that McCoy cells were more susceptible to infection with psittacosis virus than FAM cells. Strain SA-2 of trachoma virus showed this same relationship.

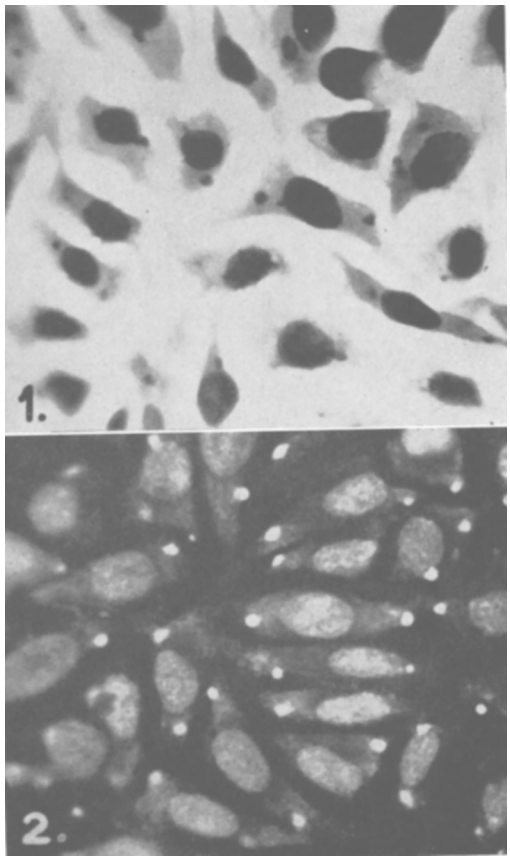

FIG. 1. McCoy cell culture infected with trachoma virus, third passage, showing inclusion bodies in cytoplasm. Wright's-Giemsa stain, ×750.

FIG. 2. McCoy cell culture infected with trachoma virus, fifth passage. With acridine orange stain and ultraviolet illumination, cytoplasmic inclusion bodies which here appear white are colored red in the original preparation, cytoplasm and nucleoli appear lighter red, and nuclei appear yellow green. ×750.

Summary. Propagation of trachoma virus (SA-2 strain) in McCoy cells was shown by 1) appearance of increased numbers of infected cells with serial passage, 2) infectivity of tissue culture fluids for embryonated chicken eggs, and 3) lack of pathogenicity for mice. The latter characteristic helps to differentiate trachoma from psittacosis viruses(2).

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Received April 11, 1960. P.S.E.B.M., 1960, v104.

Absorption and Tissue Concentrations of Vitamin B_{12} in Obese Rats. (25787)

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Bosshardt *et al.*(1) noted that growth of weanling mice on a high fat diet was markedly improved by administration of crystalline Vit. B_{12}. However, McCollum *et al.*(2) did not demonstrate any significant difference in growth of rats when Vit. B_{12} was added to diets containing different levels of fat in diet regardless of administration of Vit. B_{12}. Fox *et al.*(3) observed that the requirement for Vit. B_{12} by chicks was increased when the diet contained 22% fat, but that methionine alone could replace the increased requirement for this vitamin. They also showed that the higher Vit. B_{12} requirement was due neither to lowered absorption of dietary Vit. B_{12} nor to its subsequent storage in the liver. The involvement of Vit. B_{12} in conversion of pantothenic acid to coenzyme A has been suggested by Yacowitz *et al.*(4). In spite of these reports and the evidence presented by Ling and Chow(5) for the role of Vit. B_{12} in lipid metabolism in rats and, more recently, by Yesair *et al.*(6) in chicks, the metabolic relationship between Vit. B_{12} and lipids has not yet been established. Therefore, it is of interest to study the metabolism of this vitamin in obesity, an abnormal state of fat metabolism. The present communication presents a study of absorption and tissue concentrations of Vit. B_{12} in rats that became obese as a result of consuming a high fat diet.

Methods. Rats of the Osborne-Mendel strain were made obese by feeding *ad libitum* a diet containing 60% of fat by weight(7). They reached 1 kg of body weight in 10 to 14 months. Control rats were fed a 3% fat diet with the same composition as that of the 60% fat diet except that 57% sucrose replaced the Crisco; the control animals in earlier studies received Purina. The Vit. B_{12} concentrations in plasma and livers were determined using Skeggs' media and *Lactobacillus leichmannii* 4797 as the test organism(8). Cobalt-60 Vit. B_{12} with a specific activity of 1 $\mu c/\mu g$ was used for the absorption studies. Fifty $m\mu g$ of Co^{60}-B_{12} in 1 ml aqueous solution was administered orally through a plastic stomach tube to rats which had been fasted overnight. The feces were collected during the next 4 days, homogenized, and radioactivity was measured with a gamma-scintillation counter (9). The difference between the administered dose and amount of radioactivity recovered in the feces was considered as that which was absorbed.

Results. Vit. B_{12} levels in the plasma of obese rats were found to be higher than those in lean animals (Table I) and the differences are statistically significant. This difference is not explainable on the basis of food intake or dietary level of Vit. B_{12}, because both the 3% fat diet for control animals and the high fat diet for obese animals contained the same amount of Vit. B_{12} per gram