these data are hardly of diagnostic value, since significant changes are found usually in patients with clinically obvious disease. Our study, however, by apparently showing the effects of perchloric acid upon cholesterol-containing components of serum, different from normal to pathologic conditions, may eventually bring a new approach in the investigation of disease states with abnormalities in cholesterol metabolism. Such possibility is being studied.

Summary. 1. A fairly fixed amount of free cholesterol and cholesterol esters is found in the perchloric acid soluble (seromucoid) fraction of human serum. Phenomena of co-precipitation or of adsorption are probably responsible for this finding. 2. Seromucoid fraction, total serum cholesterol, cholesterol found in the seromucoid fraction were studied quantitatively in pathologic sera obtained from patients with lymphoma, disseminated malignancies, acute leukemia and from selected patients with liver disease. Seromucoid fraction was elevated in all diseases. Cholesterol found in the seromucoid fraction was relatively increased in sera of patients with lymphomas and metastatic carcinomas; relatively or absolutely decreased in acute leukemias and liver disease. 3. The significance of these findings consisted in the indication of possible effects of perchloric acid on cholesterol-containing components of serum. Such effects seemed to vary from normal to pathologic conditions, thus suggesting the possible future value of this study in the investigation of abnormalities of cholesterol metabolism in disease.

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Received June 19, 1957. P.S.E.B.M., 1957, v96.

Pseudorabies Virus in Tissue Culture: Differentiation of Two Distinct Strains of Virus by Cytopathogenic Pattern Induced.* (23392)

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Scherer and Syverton(1) described the cultivation of pseudorabies virus in HeLa cells. They pointed out the rounding and granulation of the infected cells, and the presence of intranuclear inclusion bodies. The present paper describes the effects of pseudorabies virus on monkey kidney cells, and the separation of 2 strains of virus on the basis of their effects on the monkey kidney cells.

Materials and methods. Virus. The rabbit brain adapted Aujeszky strain of pseudo-

* This study was made under a grant-in-aid from the United States Public Health Service.

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rabies virus was supplied by the National Type Culture Collection. Before tissue culture adaptation, 18 egg passages had been made on the chorioallantoic membrane (CAM) of 13-day-old chick embryos. Each passage showed typical pocks. After adaptation to tissue culture, virus was recovered in the supernatant fluid after centrifuging infected cultures at 3,000 *rpm* for 5 minutes. This was used immediately for most experiments; if necessary it was stored at 4°C for intervals not exceeding 48 hours.

Cell culture. The monkey kidney cell bottles were obtained from the Microbiological Associates, Inc., and maintained in a growth medium consisting of 2 parts calf serum, 98 parts of 5% lactalbumin hydrolysate in Hanks' balanced salt solution (BSS) with addition of penicillin and streptomycin in a concentration of 100 units, and 50 γ/ml, respectively. Cells were trypsinized by the addition of 20 ml of 0.5% trypsin (Difco 1:250) followed by incubation at 37°C for 20 minutes. After concentration at 900 *rpm* for 5 minutes, supernates were discarded, and sedimented cells were resuspended in fresh growth medium to yield a final concentration of 10^5 cells per ml. Aliquots of 20 ml of this cell suspension were transferred to bottles; 1 ml to screw cap tubes (Microbiological Associates, Inc.), and 5 ml to Petri dishes. Average yields of cells after 4 days at 37°C were in the range of 4 x 10^6 cells in bottle cultures, 3 x 10^5 in tubes, and 1 x 10^6 in the Petri dishes.

Virus assay. The $TCID_{50}$ (Tissue culture infecting dose) was determined in tube cultures inoculated with 0.1 ml of 10-fold dilutions of virus. The cytopathogenic effect was recorded daily for 5-7 days.

Antiserum. A swine pseudorabies virus antiserum was obtained through the courtesy of Dr. R. E. Shope. It showed no toxicity to the cell cultures.

Cytology. For detailed microscopy, cells were grown on coverslips inserted in the tubes prior to the seeding of the cells. When infected they were fixed by acetic Zenker solution, and stained with Hematoxylin-Eosin. Tube cultures were also stained with Giemsa *in situ.*

Experimental. When egg-adapted pseudorabies virus was passed serially in monkey kidney tissue cultures it was noted that different types of cytopathogenicity began to emerge. Originally the rounding and granulation of the cells, as described by Scherer and Syverton(1) were the only effects observed. However, after 3 bottle passages a few areas were noted in which small, multinucleated giant cells appeared. By a process of selection (to be described below) 2 strains of virus were separated; in one of these the predominant cytopathogenic effect was rounding and granulation of cells, and in the other giant cell formation and cytolysis. The cytologic characteristics of these 2 strains were as follows.

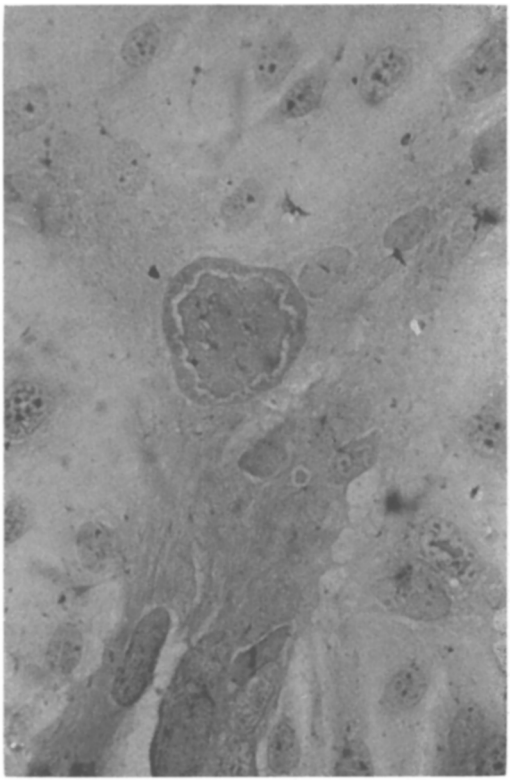
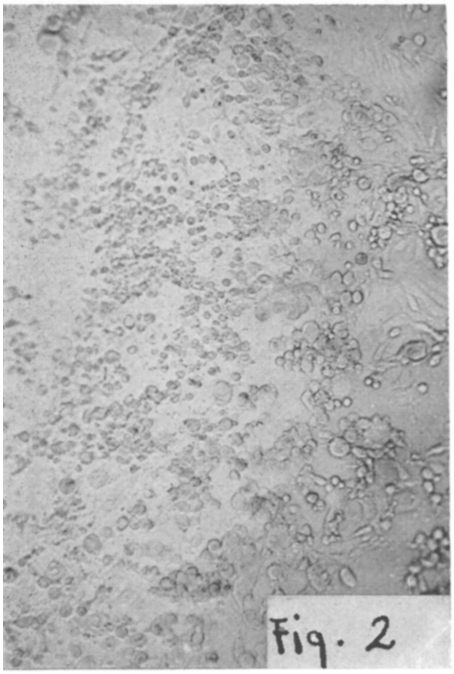
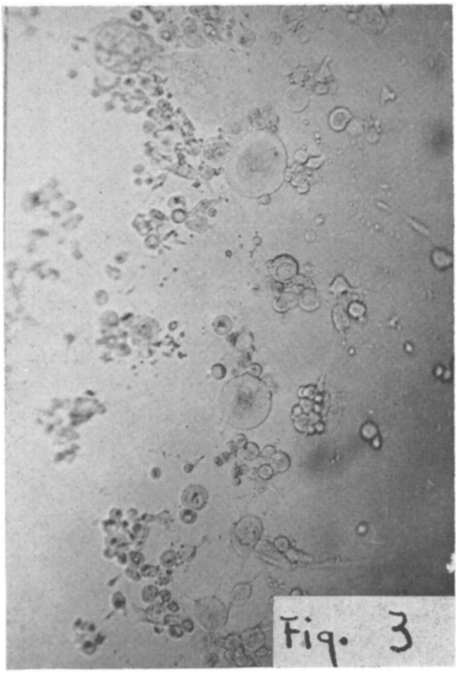


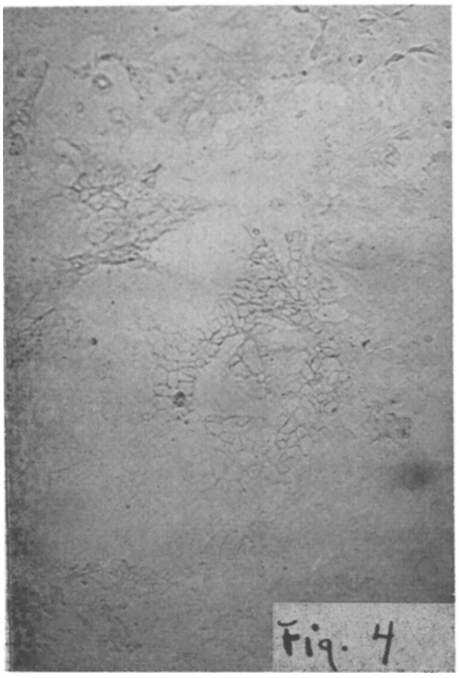
FIG. 1. Intranuclear inclusion bodies and ballooned nucleus. H & E stain × 600.

(a) *Rounding degeneration, "G" for granulation.* The initial plaque was characterized by an area of rounded and ballooned cells. Occasionally, 3-dimensional proliferation could be seen. An inspection of individual cells revealed various stages of development of type A intranuclear inclusion bodies (Fig. 1). In the earlier stages the changes in the

nucleus in which there were enlargement, margination of chromatin and presence of an inclusion body of the "full" type were similar to those described by Scott *et al.*(3) in rabbit







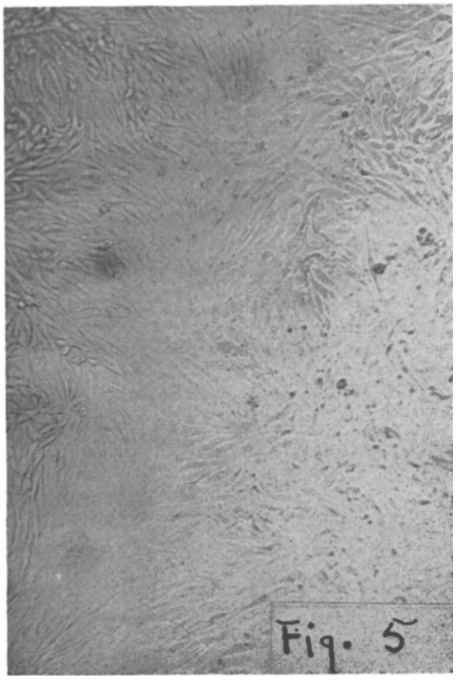


FIG. 2. Rounded cells of "G" virus, unstained × 60.
FIG. 3. Giant cells of intermediate size, unstained × 60.
FIG. 4. Aggregated ballooned nuclei with full inclusion bodies in the center of a lysed area. Cell membranes not visible, unstained × 60.
FIG. 5. Normal monkey kidney cell tube culture, unstained × 60.

TABLE I. Method of Separation of "L" Strain by Selective Limiting Dilutions.

Dilution	Passages 1	2	3	4	5	6	7
10^{-0}	GGGG	GGGG	GGGG	GGMM	GGGG	MLLL	LLLL
10^{-1}	GGGG	MMMM	MMML*	MMMM	MMMM	LLLL	LLLL
10^{-2}	MMMG	MMMM*	M000	MMML	MMMM	LL*00	0000
10^{-3}	MMMM	0000	0000	MMML*	MMML*	0000	0000
10^{-4}	GMMM*	0000	0000	GM00	0000	0000	0000
10^{-5}	GG00	0000	0000	0000	0000	0000	0000
Incubation, days	4	2	2	3	3	2	2
No. of nuclei in giant cells	8	10	30	50	Aggregated nuclei, cytolysis 100 or more		

* Used for passage.

G–G virus type; L–L virus type; M–mixed type.

corneal cells infected with herpes simplex virus. The inclusions seen in the later stage were typical type A, such as those observed by Traub in pseudorabies virus infection(4). Frequently the picture of an amitotic division of the nucleus was observed. With advancing infection, the rounded cells became necrotic and sloughed off from the glass. The changes finally involved the whole culture (Fig. 2).

(b) *Giant cells and cytolysis, "L" for lysis.* This reaction appeared to evolve with passage, and although giant cells were seldom absent altogether, they were more frequent in the earlier passages in which lysis was less marked than they were in the later passages in which lysis was the predominant characteristic. In the early passages there appeared among the rounded cells small giant cells each with 4-10 nuclei containing full inclusion bodies. These sometimes were connected to each other by protoplasmic strands (Fig. 3). In other places a giant cell formed the center of an isolated group of rounded cells.

As selective passages were made larger giant cells were observed containing 100 or more nuclei. Finally the characteristic picture consisted of a disappearance of cell boundaries leaving in the center a large aggregation of nuclei each containing an inclusion body, or the lytic effect of the virus was so great that only holes in the cell sheet were seen without even the nuclei remaining intact (Fig. 4). The appearance of normal monkey kidney cells is shown in Fig. 5.

Separation of 2 virus strains. This was accomplished by selective passage at limiting dilutions.

Table I summarizes the results of selective limiting dilutions. It can be seen that in the original material, which was derived from a bottle culture in which both types of cytopathogenicity were noted, the "G" strain predominated. A tube with this characteristic was then passed serially. This strain was found consistently to cause the characteristic rounding and granulation effect with no or only occasional giant cells for 25 passages. The "L" strain was obtained with more difficulty. Since no lysis was observed in the original material, those tubes containing a number of giant cells were used for passage. As mentioned, the giant cells in the earlier passages were small to moderate in size containing 8-10 nuclei. However, with passage larger and larger giant cells were seen. Lytic areas were first noticed in passage No. 3 in which the giant cells contained about 30 nuclei. Lysis appeared in all tubes of the 10^{-1} dilutions of passage 6, at which time no giant cells, as usually pictured, were seen; however, aggregations of more than 100 nuclei without definitive cell boundaries were seen in the midst of an area of lysis. By the seventh passage mainly lytic areas were noted. This characteristic was maintained for 15 further passages at limiting dilutions without change. Both "G" and "L" strains were neutralized by specific antiserum after a total of 25 passages in tissue culture.

It was also possible to separate these 2 strains by the plaque technic(2) using plasma clot overlay.

Growth curve. This was studied in both strains and little difference between the 2 was

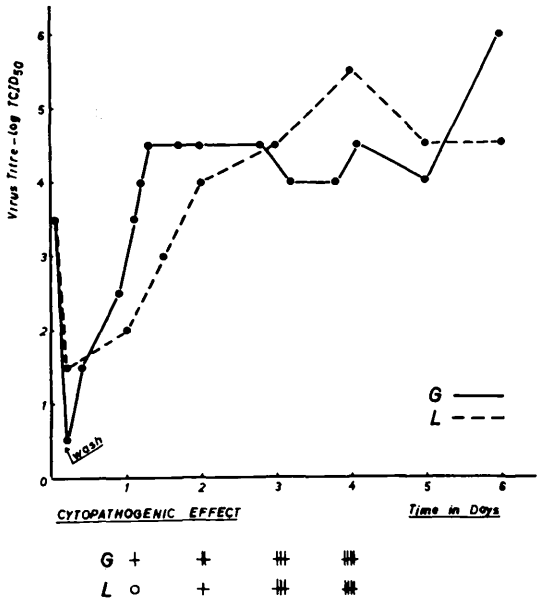


FIG. 6. Growth curve of "G" and "L" strains.

noted (Fig. 6). The highest titers were $10^{6.0}$ and $10^{5.5}$ $TCID_{50}$ respectively for the "G" and "L" strains. There was no significant difference in heat stability between the 2 strains.

Plaque Form. In order to demonstrate the difference in plaque form of the 2 strains the technic of limiting the infection to one growth cycle by means of immune serum (Black and Melnick(5)) was utilized.

One hundred plaque forming units of "G" and "L" strains of the 15th tissue culture passage were introduced into separate tube cultures. After one hour the medium was replaced with fresh growth medium containing 1:30 and 1:60 dilutions of antisera or with fresh growth medium alone. By the fourth day of incubation at 37°C the cells in the control tubes (without antiserum) of both strains had sloughed off the glass. In the tubes with 1:30 dilution of antiserum the formation of daughter foci had been completely prevented. While in the tubes with 1:60 dilution only partial prevention was obtained. The tubes with 1:30 antiserum (Fig. 7) stained with Giemsa *in situ* showed that the number of plaques was approximately equal ($\pm$ 30) for each strain, indicating a similar adsorption efficiency, but that the "L" strain plaques were large, whereas the "G" strain plaques were small.

Infectivity in rats. Pseudorabies virus causes characteristic neurological symptoms in infected rats. The animals, after an incubation period of 48-72 hours after inoculation into the eye began to scratch the skin of the face on the side of the inoculated eye. The itching becomes intolerable and the animals may tear off their skin before they die. The mechanism of these symptoms has been described by Dempsher *et al.*(6). These workers found bursts of spontaneous electrical discharges occurring first in the postganglionic fibers of the superior cervical ganglion, then in both pre- and postganglionic fibers and finally only in preganglionic fibers in animals with symptoms. It was of interest to test whether there was any correlation between the type of cytopathogenicity and the production of symptoms in rats. Preliminary experi-

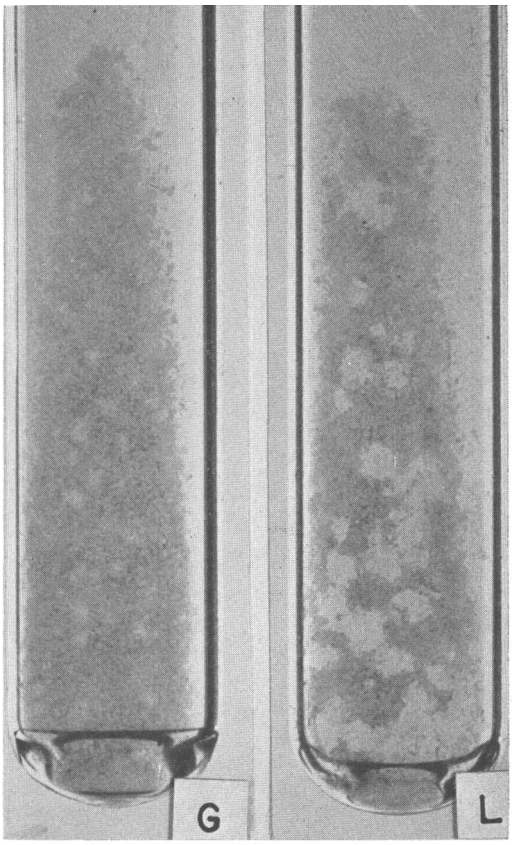


FIG. 7. Large and small plaques of "L" and "G" strains in an immune environment. Monkey kidney cell culture stained with Giemsa × 2.

TABLE II. Characterization of the Virus Strain.

	"G" strain	"L" strain
Plaque size	small	large
Cytopathogenicity	rounding cells	cytolytic giant cells
Intranuclear inclusion	positive	positive
"Mad-itch" in rat	negative or weak	"
CAM	typical pocks	typical pocks
Hemolytic activity	negative	negative
Antiserum	neutralized	neutralized

ments revealed that the "L" strain uniformly produced symptoms but these were only rarely produced by the "G" strain even though the $TCID_{50}$ titers were approximately the same.

The characteristics of the 2 strains are summarized in Table II.

Discussion. The demonstration of 2 types of cytopathogenicity in monkey kidney cells infected with pseudorabies virus suggests either that the original stock virus contained 2 strains of virus particles which could be differentially selected by adaptation in monkey kidney cells or that mutation from the "G" to the "L" strain occurred in this host. At present it has not been possible to separate these 2 strains sufficiently cleanly to test them for genotypic characteristics by recombination experiments. The apparent correlation between type of cytopathogenicity and ability to cause symptoms in rats raises the possibility that the virus which affects cell membranes in tissue culture leading to lysis may have some action on the nerve membrane which in turn gives rise to the abnormal electrical responses described by Dempsher *et al.* This point is under further investigation.

Summary. During the course of adaptation of the egg-adapted pseudo-rabies virus to tissue culture of monkey kidney cells, 2 types of cytopathogenic variations were encountered which appeared to be induced by 2 different strains of virus. One strain produced rounding degeneration, and the other cytolytic giant cell formation. Descriptions are given of the growth curve, plaque type in tissue culture, and infectivity for rats of these 2 strains.

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Received June 20, 1957. P.S.E.B.M., 1957, v96.

Influence of Estrogens on Body Growth and Food Intake.* (23393)

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Estrogens have been shown by many workers to depress body weight in laboratory animals(1-5). Several lines of evidence, too extensive to review here, indicate that they may do this by influencing either the release or action of other hormones in the body. Heavy or prolonged treatment with estrogens also causes anorexia. The resulting inanition may be partially responsible for the effects sometimes attributed to hormonal alterations. In addition, inanition itself may result in hormonal imbalances(6,7,8), which resemble in some respects the effects brought about by estrogen treatment. Therefore, dietary restriction, whether imposed experimentally or

* Supported in part by funds from the Department of Health, Education, and Welfare, Nat. Cancer Inst.

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