that platelets do carry out various metabolic activities, one may wonder about the interrelationship between their rate of metabolism and their ability to maintain their structural integrity. Is a normal rate of metabolism a prerequisite for an intact platelet structure or is the maintenance of an intact structure dependent on normal rate of metabolism? At present there is no clear-cut answer to these questions although data on effects of various other types of enzyme inhibitors on platelet stability indicate that it is more likely that platelet structure is dependent on platelet metabolism (unpublished). Thus, it is suggested, although by no means proved, that increased rate of platelet breakdown observed in presence of SH-inhibitors may be brought about by effects of these substances on a variety of SH-dependent enzyme systems essential to normal platelet metabolism.

Summary. 1. Incubation of freshly prepared human platelets with a variety of sulfhydryl inhibitors results in increased rate of platelet breakdown. 2. In all cases studied the effects of inhibitors can be decreased in extent by suitable concentrations of SH-containing substances such as cysteine or reduced glutathione. 3. Results obtained suggest a direct relationship between platelet sulfhydryl groups and platelet structure. The possible link of this relationship to platelet metabolism is briefly discussed.

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Cytopathogenic Effect Produced by Polyoma Virus in Cultures of Milk-Adapted Murine Lymphoma Cells (Strain P388 D_1).* (24582)

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Production of pleomorphic tumors of the parotid glands of mice by inoculation of newborn animals with cell-free filtrates prepared from mouse neoplasms has been described both by Gross(1) and by Stewart,(2,3) and has been confirmed in several laboratories(4,5, 6). In addition to parotid gland tumors, the inoculated mice have often developed tumors in other salivary glands, mammary glands, the thymus gland, the subcutaneous tissue and other sites. Recently, Eddy and her associates have reported that preparations of the agent also produce sarcomatous and angiomatous tumors in hamsters(7). Evidence suggesting replication of the agent in monkey kidney cell cultures was obtained, but cytopathogenic changes were not observed in most of the infected cultures(8). Subsequently, it was shown that the agent could be propagated in minced mouse embryo cultures, and cytopathogenic changes were observed with some preparations(9). Recently, Eddy and her as-

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sociates have found that the agent consistently produces cytopathogenic effects in mouse embryo cultures, and that this activity is correlated with induction of tumors in hamsters (10). Hemagglutination of guinea pig erythrocytes by the agent has also been described, and hemagglutination inhibiting antibody has been found in sera of animals inoculated with infected tissue culture fluids(11). Eddy and her associates have suggested that the agent they have studied be called SE Polyoma Virus (10,11). This paper describes a cytopathogenic effect produced by polyoma virus in cultures of a cell strain (P388 D_1) derived from a murine malignant lymphoma(12). The virus produces cellular destruction in 2 to 6 days with undiluted inocula, and virus pools prepared in P388 D_1 cell cultures have titers as high as $10^{7.5}TCID_{50}$ in 0.25 ml. The cell strain has been adapted to growth in a serum-free medium containing 20% autoclaved non-fat milk, and, therefore, the cell cultures are free of serum antibodies(13). Cultures can be inoculated with virus while in this serum-free growth medium and can be observed for long periods of time, since the control cultures do not degenerate, as usually occurs in other tissue culture virus-host cell systems in low serum concentrations or in serum-free maintenance media.

Materials and methods. Viruses. The first preparation of polyoma virus studied was obtained from Dr. Bernice Eddy and Dr. Sarah Stewart and was designated by them as "strain 210 S. E. Polyoma." It consisted of tissue culture fluid from the 35th passage of the agent in mouse embryo tissue culture and had been through 2 passages in mice and 2 passages in hamsters. In the animals, tumors had been produced as has been described by Stewart and Eddy(3,7,8,9,10). A second preparation of virus was obtained from Dr. C. J. Dawe and Dr. L. W. Law of the National Cancer Institute. This preparation was a cell-free Selas 03 filtrate of an extract of a parotid gland tumor from a $C_3H/_{Bi}$ mouse. On the day of birth this mouse had received material from a short-term tissue culture of a thymic tumor. The thymic tumor, in turn, had appeared in a $(C_3H_{fx}AKR)_{F1}$ mouse that had received, on the day of birth, material from a short-term culture of leukemic tissue from a $C_3H/_{Bi}$ mouse. The leukemia in this animal was in its eleventh serial mouse passage, and had originated in a mouse of the "Passage A" series of the experiments of Dr. Ludwik Gross. The derivation of this strain of virus and some of its biologic properties will be described in greater detail by Dawe, Law and Dunn. *Cell cultures.* The cell strain (P388 D_1) used in these studies was originally isolated by Dawe and Potter, from the 49th mouse passage of a methylcholanthrene-induced lymphoid neoplasm (P388) of a DBA/2 mouse(12). They described a morphologic alteration in tumors derived from cultivated cells as compared with the serially mouse-passaged parent tumor. The culture-derived tumors resembled reticulum cell sarcomas, and the altered cells showed marked phagocytic activity *in vitro*. The cell strain was grown in a medium containing 40% human serum up to the time we received it. To eliminate serum antibodies and serum inhibitors, we have adapted this cell strain to growth in a serum-free medium containing 20% autoclaved non-fat milk and 80% medium #199. Adaptation of these cells to the serum-free medium, preparation of the medium and methods of growing the milk-adapted cells have been described(13). In the virus experiments, full grown tube cultures of milk-adapted P388 cells containing approximately 1,500,000 cells and 2 ml of the milk medium have been inoculated with 0.25 to 0.5 ml of virus preparation. After inoculation, the cultures continue to lower pH of medium and are fed every 2 to 3 days by removing 1 cc of medium and replacing it with 1 cc of fresh medium. When the cytopathogenic effect is evident, the entire culture including both cells and supernatant fluid is frozen at –20 degrees and stored for future passage in tissue culture or in animals. Since the inoculated cultures are in serum-free growth medium, it would be possible to observe tubes in virus titrations for very long periods of time if they were fed with new medium at appropriate intervals. We have found, however, that even high dilutions of virus produce a cytopathogenic effect within 21 days, and we have usually terminated titration experiments

after 30 days. It is possible, though, that some of the tubes inoculated with high dilutions of a virus pool which did not show cytopathogenic effect at 30 days might have done so if observed for longer periods of time. *Animal inoculations.* Pregnant golden hamsters were obtained from the animal colony of the Nat. Inst. of Health. Newborn hamsters, on day of birth or one day after, were inoculated with 0.05 cc of polyoma virus grown in milk-adapted P388 D_1 cells. The preparations were not filtered and, thus, contained cellular debris and milk medium as well as the virus. The newborn hamsters were inoculated under the skin of the back and were observed daily. Complete necropsies were performed on all animals found dead.

Results. Two tube cultures of milk-adapted P388 D_1 cells were inoculated with 0.5 cc of undiluted "strain 210 S. E. Polyoma" obtained from Drs. Eddy and Stewart. Seven days after inoculation, a cytopathogenic effect was observed in the inoculated cultures. Large numbers of cells had detached from the glass surface leaving only scattered clusters of pyknotic and fragmented cells. Supernatant fluid from these cultures was passaged to new tube cultures of milk-adapted P388 D_1 cells and a similar cytopathogenic effect was noted 7 to 9 days after inoculation. The virus has now been carried through 6 consecutive passages with a total effective dilution of 10^{-14}. In the sixth passage, cytopathogenic effect was observed 4 days after inoculation. Titration of a pool of fourth passage material by serial 10-fold dilutions in milk-adapted P388 D_1 cells has shown a titer of $10^{5.5}$ $TCID_{50}$ in 0.25 ml.

After observation of a cytopathogenic effect in cultures of milk-adapted P388 cells with "strain 210 S. E. Polyoma" virus, the cell-free parotid gland tumor filtrate provided by Dawe and Law was studied in the same cell culture system. Fifteen days after inoculation of the P388 D_1 cultures with the parotid gland tumor filtrate, some questionable cytopathogenic effect was noted in the inoculated tubes and supernatant fluids from these tubes were passaged to new tubes of milk-adapted P388 D_1 cells. Six days after inoculation, cytopathogenic effect was definite in the tubes of this second passage. The cytopathogenic effect was similar to that seen in tubes inoculated with the "strain 210 S. E. Polyoma." Subsequently, this agent has been carried through 11 consecutive passages with a total effective dilution of 10^{-21}. A pool of eleventh passage material produces a cytopathogenic effect in 2 days with undiluted inocula. Titration of this pool by serial 10-fold dilutions in milk-adapted P388 D_1 cells shows a titer of $10^{7.5}$ $TCID_{50}$ in 0.25 ml. Inoculation of tube cultures with 5 $TCID_{50}$ of agent caused cytopathogenic effects in 15 to 21 days. A tube culture inoculated with 5 $TCID_{50}$ and incubated until the cytopathogenic effect was evident was found to contain more than 8,000,000 $TCID_{50}$.

Cytopathology. Morphologically, cultures of P388 D_1 cells differ from HeLa cells and the other epithelial-like cell strains commonly used in virus studies. Instead of the confluent cell sheet seen in control cultures of HeLa, KB and similar cell strains, fully populated cultures of P388 D_1 contain large numbers of closely approximated, round, oval and somewhat elongated cells (Fig. 1). Scattered

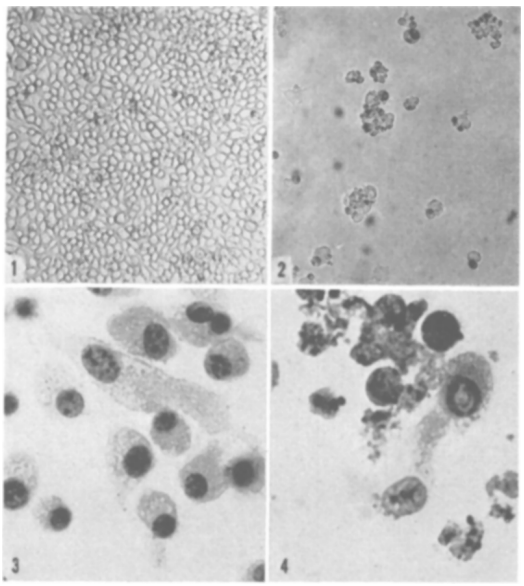

FIG. 1. Control culture of milk-adapted P388 D_1 cells. Unstained, ×70.

FIG. 2. Cytopathogenic effect produced by polyoma virus in culture of milk-adapted P388 D_1 cells. Unstained, ×70.

FIG. 3. Control culture of milk-adapted P388 D_1 cells. Papanicolaou stain, ×415.

FIG. 4. Cytopathogenic effect produced by polyoma virus in culture of milk-adapted P388 D_1 cells. Papanicolaou stain, ×415.

clumps of cells which have become heaped upon one another during growth are seen, and multinucleated giant cells are found in variable numbers.

The earliest evidence of a cytopathogenic effect in cultures of milk-adapted P388 D_1 cells inoculated with polyoma virus is the appearance of scattered small collections of necrotic cells. These cells are shrunken, distorted and more refractile than normal P388 D_1 cells. After these necrotic foci are observed, the cytopathogenic effect usually proceeds rapidly and, in 24 hours, only scattered clusters of necrotic cells remain adherent to the dependent surface of the tube (Fig. 2). Ultimately, all the cells are detached from the surface of the tube. It has not been possible to regrow a cell population in an inoculated culture by feeding fresh medium after the cytopathogenic effect has been observed.

Cultures inoculated with "strain 210 S. E. Polyoma" and cultures inoculated with the agent obtained from Dawe and Law have shown identical cytopathology.

Coverslip cultures inoculated with polyoma virus have been fixed and stained in various stages of the cytopathologic process. In cultures in which the cytopathology has been permitted to proceed to a point where most of the cells have become detached from the coverslip, scattered clumps of cytoplasmic and nuclear debris remain, with occasional clusters of cells with karyorrhexis (Fig. 4). Cells with nuclear enlargement, margination of chromatin along the nuclear membranes, and central clear areas in the nuclei are also seen. Small clumps of eosinophilic material can be seen in some of the clear areas in the nuclei in coverslips stained with H. and E. In the cytoplasm of some cells of cultures with cytopathogenic effect, phagocytized cellular detritus and necrotic cells are seen.

Animal Inoculations. Newborn hamsters have been used to evaluate the oncogenic properties of polyoma virus prepared in cultures of milk-adapted P388 D_1 cells. Four litters of hamsters were inoculated with 6th passage material derived from the mouse parotid gland tumor filtrate received from Dawe and Law. The total effective dilution of the parotid gland material in this 6th passage was approximately 10^{-20}. Forty-two animals were inoculated and all were dead within 40 days of inoculation. Since many of the dead animals were lost due to cannibalism by cage mates, only 19 of the 42 were examined at necropsy. All of these animals had multiple sarcomatous and angiomatous lesions involving heart, kidneys, liver and other sites, which grossly and microscopically are similar to the lesions described by Eddy and her associates in hamsters inoculated with polyoma virus(7). Inoculation of newborn hamsters with "strain 210 S. E. Polyoma" passaged three times in milk-adapted P388 D_1 cells has also resulted in production of similar lesions in the treated animals.

The culture fluids used for animal inoculation were not cell-free filtrates and did contain necrotic P388 D_1 cells which had been frozen and thawed once prior to injection into the hamsters. None of the tumors seen in the hamsters resembled the P388 D_1 malignant lymphoma histologically and there is no reason to suspect that the sarcomatous and angiomatous tumors we have observed represent transplantation of P388 D_1 cells to the hamsters. A control litter of 11 newborn hamsters has been inoculated with a suspension of P388 D_1 cells without polyoma virus after one cycle of freezing and thawing and no tumors have been observed in these animals 3 months after inoculation.

Discussion. The virus-host cell system of polyoma virus in milk-adapted P388 D_1 cells here described is of interest for several reasons. Rapid production of a cytopathogenic effect makes the system suitable for titrations and growth studies of the agent. Since the cell strain grows in serum-free medium, it is possible to study infection during growth of the cell population in the absence of serum antibody and serum viral inhibitors. The possible presence of inhibitors of polyoma virus in the milk medium, however, has not been determined.

Only a few reports have been found in which viruses were studied in cultures of cells of the histiocyte-macrophage-reticulum cell family, and in none of these was a generalized cytopathogenic effect noted(14,15,16). Cells of the P388 D_1 culture strain morphologically

resemble atypical histiocytes or reticulum cells, and retain their phagocytic properties as well as their ability to grow into ascites neoplasms when inoculated intraperitoneally into DBA/2 mice. The fact that polyoma virus causes cytopathogenic effects in the cells of such cultures suggests that P388 D_1 cells might be of interest in studies of other viruses.

Summary. A cytopathogenic effect has been observed in cultures of murine malignant lymphoma cells (strain P388 D_1) inoculated with polyoma virus. There is evidence of viral multiplication in these cell cultures and the virus retains its ability to produce tumors when inoculated into newborn hamsters. The virus-host cell system of polyoma virus and milk-adapted P388 D_1 cells seems to be a suitable one for studies of this oncogenic agent.

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Serum Proteins in Aminopterin Treated Rats. (24583)

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Importance of folic acid in metabolism of proteins and nucleoproteins has been recently reported. That folic acid is involved in purine and creatine metabolism, has been shown by Dinning and Day in monkeys(1) and Totter *et al.*(2) in chicks. A decrease in incorporation of C^{14} formate in nucleic acid(3) and in liver and visceral protein(4) in folic acid-deficient rats implicates this vitamin in biosynthesis of proteins and purines. Bakerman and coworkers(5,6) observed an increase in excretion of aminoacids, mainly glutamic acid, in folic acid deficiency, while Ricceri (7) observed a fall in excretion of uric acid and allantoin. Similar studies on blood have been reported by Tentori and Vivaldi(8). These observations indicate that folic acid is intimately related to protein metabolism. The present investigation was undertaken to study whether folic acid deficiency would influence the level of total serum protein or any of its electrophoretic components in rats. Fibrinogen content of plasma of folic acid deficient and pair-fed normal controls was also studied.

Methods. Adult male rats, weighing 180-240 g were fed mineralized whole milk with 2 drops of a concentrate of Vit. A and D, twice a week. Animals which grew well were paired and housed in individual metabolism cages. Folic acid deficiency was produced in one of the pairs by intraperitoneal injection of aminopterin (25 γ/day). The other rat of the pair served as control and was fed the same amount of milk as its deficient counterpart. Within 10 days of aminopterin injection rats showed the typical deficiency syndrome. Porphyrin appeared around eyes and

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