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.

1. Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 414.
2. Stewart, S. E., *Anat. Rec.*, 1953, v117, 532.
3. ———, *J. Nat. Cancer Inst.*, 1955, v14, 1391.
4. Dulaney, A. D., *Cancer Research,* 1956, v16, 877.
5. Hays, E. F., Beck, W. S., *ibid.*, 1958, v18, 676.
6. Law, L. W., Dunn, T. B., Boyle, P. J., *J. Nat. Cancer Inst.*, 1955, v16, 495.
7. Eddy, B. E., Stewart, S. E., Young, R., Mider, G. B., *ibid.*, 1958, v20, 747.
8. Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., Grubbs, G. E., *Virology,* 1957, v3, 380.
9. Stewart, S. E., Eddy, B. E., Borgese, N. G., *J. Nat. Cancer Inst.*, 1958, v20, 1223.
10. Eddy, B. E., Stewart, S. E., Berkeley, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 848.
11. Eddy, B. E., Rowe, W. P., Hartley, J. W., Stewart, S. E., Huebner, R. J., *Virology,* 1958, v6, 290.
12. Dawe, C. J., Potter, M., *Am. J. Path.*, 1957, v33, 603.
13. Rabson, A. S., Legallais, F. Y., Baron, S., *Nature,* 1958, v181, 1343.
14. Barski, G., *Science,* 1957, v125, 448.
15. Benedict, A. A., McFarland, C., *Nature,* 1958, v181, 1742.
16. Sabin, A. B., *Br. J. Exp. Path.*, 1935, v16, 158.

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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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TABLE I. Hematological Picture in Rats.

	Hemoglobin, g/100 ml (12)	Hematocrit value, % (5)	Leucocytes, mm³ (4) (× 1000)
Normal	15.51 ± .22*	47 ± .5	6.33 ± .37
Aminopterin treated	10.42 ± .27	30 ±2.0	.60 ± .14

* Mean ± S.E.
Figures in parentheses indicate No. of animals.

TABLE II. Plasma Fibrinogen Values in Rats, g/100 ml.

	Plasma fibrinogen (6)
Normal	.21 ± .02
Aminopterin treated	.55 ± .06

nose, diarrhoea was evident, the animals became inactive with loss of appetite and significant weight loss. In the evening, food was withdrawn from cages and the next morning both deficient and pair-fed normal animals were anaesthetized with pentobarbital sodium. The left carotid was exposed and a fine needle tied into it. The needle was connected to a 15 cc rubber stoppered centrifuge tube. With the help of slight suction, the blood immediately collected in the tube which was allowed to clot for serum. A small quantity of blood was taken in an oxalated tube. From this, hematological findings and samples were taken and the rest centrifuged without delay for plasma. Vitamin deficiency was established by leucocyte count and determinations of hemoglobin and packed cell volume. Total nitrogen and non-protein nitrogen of serum and plasma fibrinogen were determined by methods described previously(11). Serum proteins were separated by paper electrophoresis. 0.01 cc undiluted serum was used and electrophoresis continued for 16 hrs with a current of 4 mA and 200 volts in barbiturate-barbituric acid buffer of pH 8.6 and ionic strength 0.05. Staining of paper strips, determination of optimum density curves of the coloured zones by densitometry and calculation of amounts of different fractions of serum proteins were the same as described previously(11).

Results. Results are given in Tables I, II and III. A low hemoglobin content, diminution in number of white blood corpuscles and a low hematocrit value were noted in aminopterin-treated rats (Table I). The serum protein pattern of these animals was characterized by lowering of albumin and γ-globulin and increase in α 2-globulin values. These values have significance at the p 0.01 level. There was no significant change in α_1 and β-globulin fractions. There was a decrease in total serum protein, but a significant increase in fibrinogen content in aminopterin induced folic acid deficient animals when compared to normal controls.

Discussion. Folic acid is reportedly involved in nucleoprotein synthesis(3) and ribonucleoprotein is probably related to protein synthesis(12). It is possible that in animals deficient in folic acid formation of nucleoprotein and hence protein synthesis is retarded(4). This is a possible explanation of our finding of decreased serum protein and albumin. Since both fibrinogen and albumin are made in the liver the rise in fibrinogen indicates that aminopterin interference with liver function is not a simple depression of protein synthesis(13,14). The present findings with aminopterin treatment are similar to those with ascorbic acid deficiency(9). This may be because metabolism of folic acid and ascorbic acid are closely interrelated(15), or because this is the usual response of the liver to injury (Ed.).

The electrophoretic pattern of serum globulins with aminopterin treatment is not similar to that of combined folic acid and Vit. B_{12} deficiency reported by Mulgaonkar and Sreeni-

TABLE III. Different Fractions of Serum Proteins in Rats (%).

Rats	Albumin	Globulins				A/G ratio
		α_1	α_2	β	γ	
Normal (12)	2.06 ± .07	1.20 ± .06	.41 ± .02	1.20 ± .05	1.12 ± .06	.52
% of total protein	34.39	20.03	6.85	20.03	18.70	
Aminopterin treated (12)	.83 ± .05	1.14 ± .08	.84 ± .05	1.18 ± .07	.43 ± .04	.23
% of total protein	18.78	25.79	19.00	26.70	9.73	

vasan(10). Our values with normal rats, however, compared favourably with theirs. In their case a decrease in α_1-globulin with significant increase in β-globulin was observed, whereas under our conditions an increase in α_2-globulin with a decrease in γ-globulin was observed.

Summary. Different fractions of serum proteins were determined by paper electrophoresis in folic acid deficient and pair-fed normal rats. Total serum protein was low and plasma fibrinogen was significantly high in the deficient animals. There was a significant decrease in albumin and γ-globulin, considerable increase in α_2-globulin, with no significant change in α_1 and β-globulin fractions in the serum of folic acid deficient rats.

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1. Dinning, J. S., Day, P. L., *J. Biol. Chem.*, 1949, v181, 897.

2. Totter, J. R., Kelly, B., Day, P. L., *ibid.*, 1950, v186, 145.

3. Skipper, H. E., Mitchell, J. H., Benett, L. L., *Cancer Research*, 1950, v10, 510.

4. Plant, G. W., Betheil, J. J., Lardy, H. A., *J. Biol. Chem.*, 1950, v184, 795.

5. Bakerman, H. A., *ibid.*, 1951, v188, 117.

6. Silverman, M., Gardiner, R. C., Bakerman, H. A., *ibid.*, 1952, v194, 815.

7. Ricceri, G., *Boll. Soc. ital. biol. sper.*, 1956, v32, 277.

8. Tentori, L., Vivaldi, G., *Rend. ist. super. sanita.*, 1953, v16, 814.

9. Banerjee, S., Rohatgi, K., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 234.

10. Mulgaonkar, A. G., Sreenivasan, A., *ibid.*, 1957, v94, 44.

11. Banerjee, S., Chatterjee, K. P., *ibid.*, 1957, v94, 474.

12. Demsey, E. W., Wislocki, G. B., *Phys. Rev.*, 1946, v26, 1.

13. Sterling, K. S., *Clin. Invest.*, 1949, v28, 1057.

14. Brante, G., *Scand. J. Clin. Lab. Invest.*, 1952, v4, 293.

15. Lahiri, S., Banerjee, S., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 545.

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Exchange of Radioactive Magnesium in the Rat.*† (24584)

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Changes in extracellular concentration of magnesium have been implicated in various pathological states and in mammalian hibernation. The clinical aspects have been reviewed by Flink(1) and Elkinton(2) and the role of magnesium in hibernation by Riedesel(3). The recent availability of a radioactive isotope, Mg^{28}, has provided a tool for investigating the dynamic equilibrium between extracellular magnesium and that in the cells and/or skeleton. The present report concerns the exchange of magnesium between plasma and soft tissues in the rat.

Methods. Mg^{28} is an isotope emitting both gamma and beta radiation with a half-life of 21.3 hours. It decays to Al^{28} which also emits gamma and beta radiation with a half-life of 2.3 minutes, and this daughter decays to stable Si^{28}. The isotope, with Mg^{24} carrier, was obtained from the Brookhaven National Laboratory and was prepared to yield a solution containing 150 meq/l magnesium. Twenty-six male rats ranging in weight from 290 to 325 g were each injected with about 0.5 ml of this solution intraperitoneally. The injections were made from a tuberculin syringe which was weighed on an analytical balance before and after each injec-

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