experiments may be difficult to interpret and reproduce.

Our unexpected finding with SV15, as to its resistance to 1M $MgCl_2$, suggests the possibility that other DNA viruses, reported to be inactivated by 1M $MgCl_2$(4,5,6), may also demonstrate a similar dependence upon pH as a requirement for inactivation. These studies are now being carried out.

Summary. When Simian adenovirus Type 15 was heated at 50°C in the presence of 1M $MgCl_2$, it was found that inactivation of the virus infectivity was dependent upon the pH of the test medium. Dilution of dialyzed virus in 0.05M L-histidine buffer at pH 5.0 or 5.4 resulted in no inactivation of the virus following 2 hours' heating at 50°C in 1M $MgCl_2$; however, virus inactivation increased in response to an increase in pH from 5.7 to 6.9 under the same test conditions.

We wish to thank Helen P. McCray for technical assistance and Drs. Ivy R. McManus and Julian Andelman for helpful discussions.

1. Wallis, C., Melnick, J. L., Texas Rep. Biol. and Med., 1961, v19, 683.

2. ———, Virology, 1962, v16, 504.

3. Melnick, J. L., Ann. N. Y. Acad. Sci., 1962, v101, 331.

4. Wallis, C., Melnick, J. L., Texas Rep. Biol. and Med., 1961, v19, 701.

5. Wallis, C., Yang, C.-S., Melnick, J. L., J. Immunol., 1962, v39, 41.

6. Petek, M., Felluga, B., Zoletto, R., Bersani, G., Arch. ges. Virusforsch., 1964, v14, 637.

7. Atchison, R. W., Casto, B. C., Hammon, W. McD., Science, 1965, v149, 754.

8. Wallis, C., Lewis, R. T., Melnick, J. L., Texas Rep. Biol. and Med., 1961, v19, 194.

9. Melnick, J. L., Wallis, C., Proc. Soc. Exp. Biol. and Med., 1963, v112, 894.

Received May 23, 1966. P.S.E.B.M., 1966, v122.

Virus Particles in Hamster Tumors as Revealed by Electron Microscopy.* (31365)

W. A. STENBACK, G. L. VAN HOOSIER, JR., AND J. J. TRENTIN
Division of Experimental Biology, Baylor University College of Medicine, Houston, Texas

A group of 11 different hamster tumors, maintained by transplantation, were surveyed by thin section electron microscopy for virus particles. This group contained one tumor of spontaneous origin and some which arose after inoculation of viral agents into newborn hamsters of our closed colony. Two of the tumors arose in other colonies and were imported and maintained in a separate "open" colony. These are the Eddy fibrosarcoma induced by SV40 and the non-viral Fortner sarcoma No. 3B. The present report deals with preliminary positive findings of particles, some of which resemble murine leukemia agents and some of which appear identical with particles reported by Bernhard and Tournier(1) in tissue cultures of hamster cells and hamster tumors.

* This investigation was supported in part by USPHS Research Grants CA-06941, CA-05021, and K6-CA-14219 from Nat. Cancer Inst.

Materials and methods. Pieces of fresh tumors were excised, minced in cold 8% glutaraldehyde and transferred to fresh glutaraldehyde for 1-2 hours. The tissues were next washed in phosphate-buffered saline (PBS) and post-fixed with 1% phosphate-buffered O_8O_4(2) for 1 hour. They were then dehydrated in graded alcohols, embedded in Araldite resin and thin-sectioned with a Porter-Blum MT-2 ultramicrotome equipped with glass knives. Sections were mounted on Parlodion-coated, carbonized grids and double-stained with uranyl acetate and lead citrate(3) for 5 minutes each. The uranyl acetate stain was prepared as a 2% aqueous solution with the pH adjusted to a point just short of precipitation (about 4.8) with 10 N NaOH.

Sections were examined in Siemens Elmiskop I or IA electron microscopes, and magnifications were calibrated with a grating replica of 54,864 lines per inch. Measurements were made with a dissection microscope equipped with a Filar micrometer eyepiece.

Results. Nine of the 11 tumors surveyed contained virus-like particles. Particles resembling murine leukemia viruses, examples of which are shown in Figs. 1 and 2, were found in 7 of the tumors. Immature type C particles, frequently in the process of budding, were the predominant forms seen. These particles measured 99 mμ average diameter with an inner electron-dense shell of 54 mμ diameter. Intermediate membranes were evident in these immature forms. Mature type

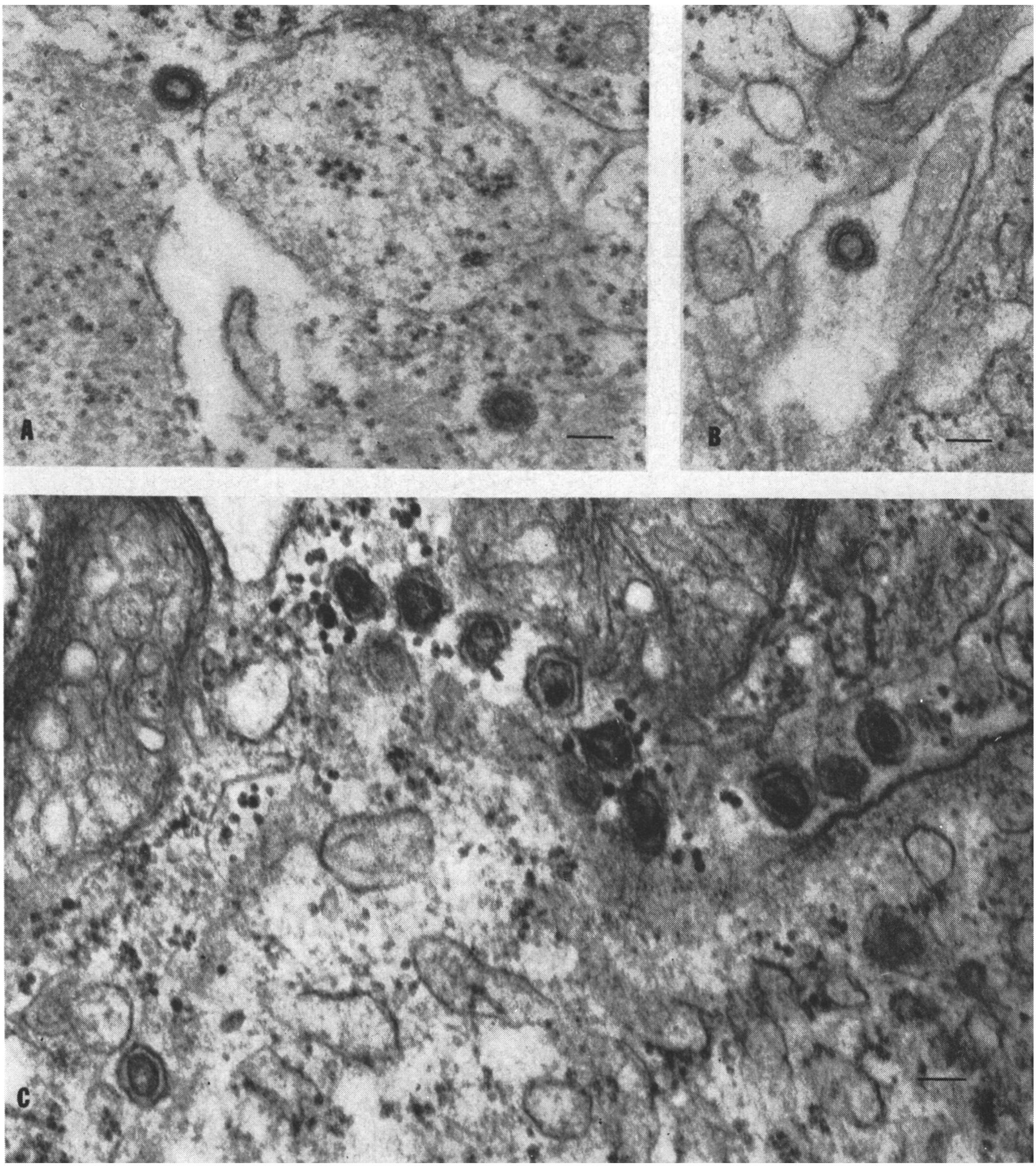

FIG. 1. Examples of murine leukemia-like agents found in hamster tumors. A and B: Immature type C particles each showing electron-translucent nucleoid and prominent intermediate membrane. C: Mature type C particles with electron-dense nucleides as seen in intercellular spaces of one tumor. The bar in all micrographs represents 100 mμ.

C particles were found in only one tumor. They were also found in high speed blood plasma pellets of animals bearing one of 3 other tumors so examined. The leukemia-like particles were found singly, or in pairs or small groups, in intercellular spaces or free within the cytoplasm of tumor cells, not associated with any particular cell organelles.

A second type of particle, examples of which are shown in Fig. 2, was found in 7 out of the 11 tumors. These particles were always associated with cytoplasmic vacuoles, and measured 98 mμ average outer diameter with small nucleoids of 30 mμ diameter. Usually an intermediate membrane was evident immediately surrounding the nucleoid, and the particles displayed radial "wheel spoke-like" rays characteristic of particles de-

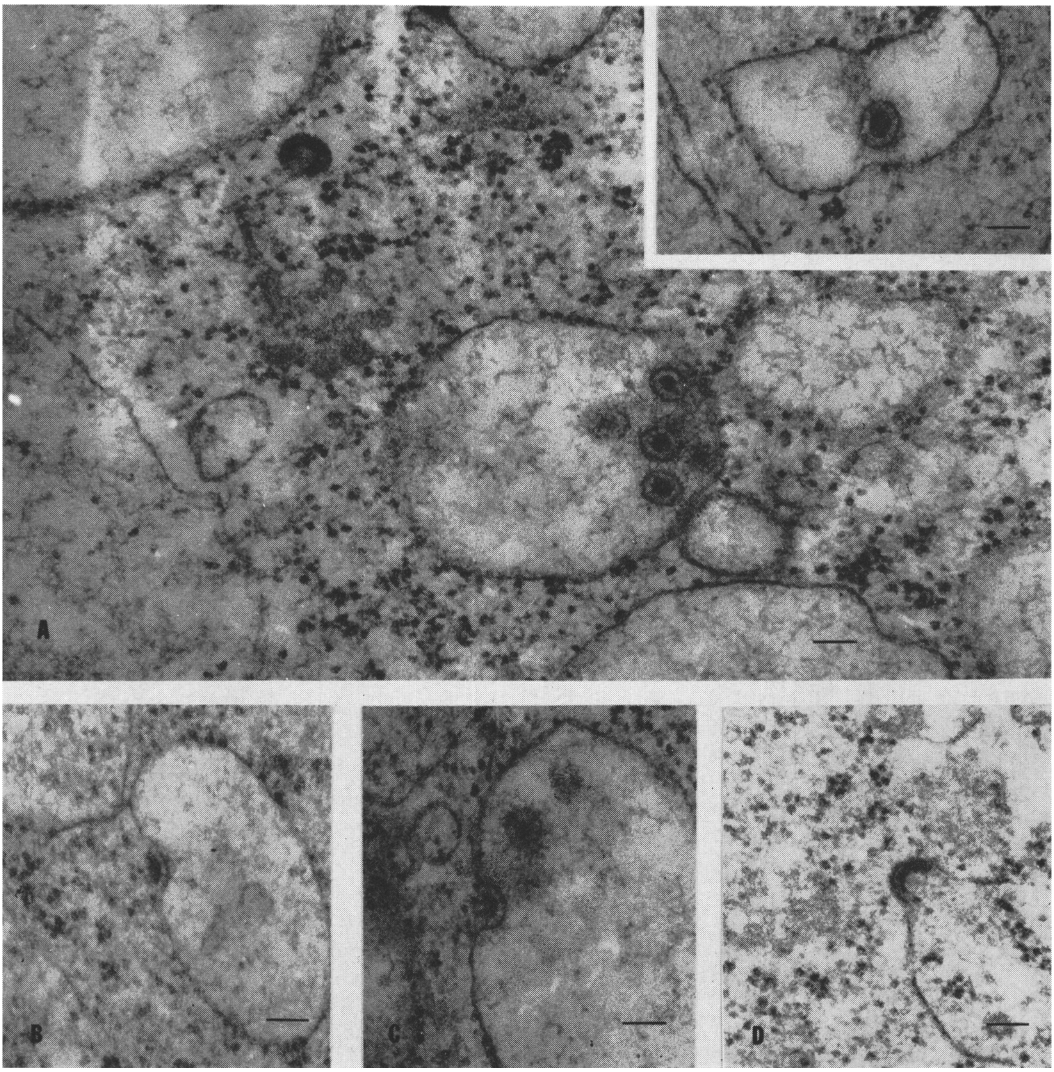

FIG. 2. Examples of the 2 types of virus particles found in hamster tumors. A: Both types of particles are present in this micrograph for comparison; a murine leukemia-like particle is at the upper left and 3 "Bernhard" type particles are in the central vacuole. Inset shows a "Bernhard" type particle with its characteristic small nucleoid, closely spaced intermediate membrane and "wheel spoke" rays in clear detail. B and C: "Bernhard" type particles apparently budding into cytoplasmic vacuoles. D: Immature type C leukemia-like particle budding from an ill-defined membrane which appears to be within the cytoplasm. Alternatively, the bud may simply be surrounded by cytoplasmic components released by cell damage.

TABLE I. Histological Type of Tumor and Associated Virus Particles Found.

Type of tumor	Treatment of hamster of tumor origin	Type of virus particle: Murine leuk.-like	Type of virus particle: Bernhard
1. Malignant lymphoma (unclassified as to type)	Adenovirus	+	+
2. "	"	+	+
3. "	"	+	—
4. "	"	+	—
5. Malignant lymphoma (reticulum cell type)	None	+	+
6. Undifferentiated carcinoma	Adenovirus	+	+
7. Fibrosarcoma (Eddy)	SV40	+	+
8. Undifferentiated sarcoma	Adenovirus	—	+
9. Sarcoma No. 3B (Fortner)	Sodium cholate	—	+
10. Undifferentiated malignant neoplasm	Adenovirus	—	—
11. "	"	—	—

scribed by Bernhard and Tournier(1). Two of the 9 positive tumors contained only the murine leukemia-like particle, while 5 of the tumors contained both types. Two tumors of the total of 11 showed no virus particles.

Table I presents the histologic diagnosis of tumors together with the types of particles found by electron microscopy. Spleen and thymus tissues from one animal (No. 1) bearing a virus-positive malignant lymphoma were examined. No virus particles were found. High-speed pellets of virus-positive tumor homogenates contained particles of the same type seen in the tumor sections, but the numbers of particles were relatively low.

Discussion. The one tumor in the group that arose spontaneously in an untreated control of our closed colony was a malignant lymphoma and contained both types of virus particles. Malignant lymphomas have been reported to arise spontaneously in untreated hamsters of other colonies(4,5). Our closed hamster colony originated from breeding stock derived from the polyoma virus-free colony of the National Institutes of Health. We have taken precautions to isolate this hamster colony from all other local animal colonies including mouse colonies(6). Tests of the sera of these hamsters for antibodies to polyoma virus have been negative. Those tumors that arose in virus-treated animals did not all contain virus particles. In those that did, the particles found and described herein do not resemble the viruses with which the original tumor host animal was treated, *i.e.*, adenovirus and SV40. Since the viruses found by electron microscopy do not consistently relate to the treatment of the tumor host in which they are found, it seems possible that they are endemic in our hamster colony and could potentially become a passenger in any transplanted tumor carried therein. This does not preclude the possibility that they are etiologically related to some of the tumors. The association of non-oncogenic infectious agents with experimental tumors is well documented, but neoplastic agents have also been isolated from transplantable tumors even though they bore no obvious relationship to the tumors(7). Since one of the particles in our series resembles murine leukemia virus and occurs in all of the lymphomas, it is possible that this agent is a hamster lymphoma virus.

To our knowledge no agent resembling a murine leukemia virus has heretofore been reported in hamsters. The particle described by Bernhard and Tournier(1) is apparently widely distributed as a latent agent in hamster tissues but differs morphologically from typical murine leukemia virus.

Summary. Examination of 11 transplantable hamster tumors by electron microscopy revealed virus particles in 9. Seven of the tumors contained particles morphologically identical with murine leukemia viruses. Five of these tumors also contained another type of particle which appears to be identical with one reported by other workers. Two of the tumors contained only the murine leukemia-like agent, and two contained only the second agent.

The authors gratefully acknowledge Dr. H. J. Spjut, Dept. of Pathology, for examination of histological sections, and Mrs. Bernie Powell and Miss Judy Chesner for technical assistance.

1. Bernhard, W., Tournier, P., Ann. de l'Inst. Pasteur, 1964, v107, 447.

2. Millonig, G., Proc. 5th Internat. Congress for Electron Microscopy, Academic Press, 1962, v2, 8.

3. Reynolds, E. S., J. Cell. Biol., 1963, v17, 208.

4. Fortner, J. G., Cancer Res., 1961, v21, 1491.

5. Weinberger, M. A., Banfield, W. G., J. Nat. Cancer Inst., 1965, v34, 459.

6. Trentin, J. J., Van Hoosier, G. L., Jr., Shields, J., Stephens, K., Stenback, W. A., Parker, J. C., Nat. Cancer Inst. Monograph 20, 1966, p147.

7. Stansly, P. G., Prog. Exp. Tumor Res., F. Homburger, ed., Hafner Publ. Co. Inc., N. Y., 1965, v7.

Received May 9, 1966. P.S.E.B.M., 1966, v122.

Effect of the Cholesterol Biosynthesis Inhibitor AY-9944 on Serum Lipoproteins of Rats, Pigs and Dogs. (31366)

P. HILL AND D. DVORNIK (Introduced by W. T. Rockhold)
(With the assistance of Patricia Ford)
Department of Biochemistry, Ayerst Research Laboratories, Montreal, Canada

The agent *trans*-1,4-*bis*(2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (AY-9944)(1) inhibits the biosynthesis of cholesterol by interfering with the enzymatic transformation of 7-dehydrocholesterol to cholesterol(2,3). Given orally to laboratory animals, *e.g.*, in rats, AY-9944 significantly lowers serum cholesterol and—in spite of accumulation of the intermediate 7-dehydrocholesterol biosynthesized instead of cholesterol—also causes reduced total sterol levels in the serum(4). In view of its potency, it was of interest to investigate the distribution of sterols and phospholipids in the serum lipoproteins of rats, pigs and dogs treated with AY-9944(5).

Methods. Albino or hooded rats, weighing approximately 120 g, were given orally 10 μmoles (4.6 mg)/kg/day of AY-9944 for 7 days; to facilitate analysis the sera from 5 rats were pooled. Young, 4-5-week-old male pigs received *per os* 5, 10 and 50 μmoles (2.3, 4.6 and 23.2 mg)/kg/day of AY-9944 for 12 weeks; individual blood samples were taken after 20 and 104 days. In another study, young pigs of the same age were given orally 25 μmoles (11.6 mg)/kg/day of AY-9944 for 28 days. Treatment was stopped for 10 days and was then repeated for 18 days. Individual blood samples were taken on days 14, 28, 38 and 56. Young beagle hounds were administered orally either 25 (11.6 mg) or 50 μmoles (23.2 mg)/kg/day of AY-9944; a third group received daily 50 μmoles/kg of the agent and the dose was increased every month by 10 μmoles/kg/day until a final dose of 110 μmoles/kg/day was reached. AY-9944 was given for 5 days per week and treatment was continued for a total of 104 weeks. In a second study in dogs, 5-8-year-old beagle hounds were given orally 30 μmoles (13.9 mg)/kg/day of AY-9944 for 28 weeks.

The serum lipoproteins were separated by preparative ultracentrifugation(6) into fractions of very low density (VLD; $d<1.019$), low density (LD; d, 1.019-1.063) and high density (HD; d, 1.063-1.21) respectively. Cholesterol and 7-dehydrocholesterol were determined as reported earlier(7) and the phospholipid levels by the semi-automated procedure of Kraml(8).

Results. Rats (Table I). Oral administration of 10 μmoles/kg/day of AY-9944 caused a fall in serum cholesterol which was partly compensated by 7-dehydrocholesterol. The effect was greater in hooded rats, notably in females, than in albinos. Cholesterol was reduced in all lipoprotein fractions. 7-Dehydrocholesterol predominated in HD lipoproteins and was not detected in the LD fraction. In spite of different sterol levels in male hooded and albino rats, their 7-dehydrocholesterol:cholesterol ratios in the VLD and HD lipoproteins were virtually identical (Table II).

Pigs. As presented in Table III, administra-