(10-100 mg intramuscularly) increased serum cholesterol of the intact rat but had no effect on the adrenalectomized rat(17). Our findings cannot be explained by a difference in food intake, as the intact rats and adrenalectomized rats with DCA, both showed a decrease in food intake during cortisone therapy (18). Since serum sodium and potassium levels were within normal limits in every group, the results cannot be explained by a change in the dilution of plasma.

The treatment of adrenalectomized dogs with DCA lowered their blood phospholipids (19). In our experience, the serum lipid phosphorus of adrenalectomized rats maintained with DCA was slightly but not significantly lower than normal.

Although the cholesterol concentration of the liver was not changed in any group, the liver lipid phosphorus concentration was increased in the adrenalectomized rats maintained with DCA. This finding is difficult to interpret.

*Summary.* 1. Cortisone therapy increased serum cholesterol and lipid phosphorus of intact rats but did not have this effect in adrenalectomized rats maintained with DCA. 2. Adrenalectomized rats maintained with DCA showed a significant increase in liver lipid phosphorus. This was restored to normal by a dosage of cortisone which was without effect on the liver lipid phosphorus of the intact rat. 3. Cortisone therapy decreased testicular weight of intact and adrenalectomized rats, but caused no change in the concentrations of cholesterol and lipid phosphorus of the testes.

1. Adlersberg, D., Schaefer, L. E., and Drachman, S. R., *J. Clin. Endocrinol.*, 1951, vII, 67.

2. Conn, J. W., Vogel, W. C., Louis, L. H., and Fajans, S. S., *J. Lab. Clin. Med.*, 1950, v35, 504.

3. Winter, C. A., Silber, R. H., and Stoerk, H. C., *Endocrinology*, 1950, v47, 60.

4. Hoffmeyer, J., *Acta Physiol. Scand.*, 1945, v10, 31.

5. Kobernick, S. D., and More, R. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 602.

6. Rich, A. R., Cochran, T. H., and McGoon, D. C., *Bull. Johns Hopkins Hosp.*, 1951, v88, 101.

7. Diczfaluzy, *et al.*, *Acta Endocrinologica*, 1950, v5, 43.

8. Salva, G., *et al.*, *Lancet*, 1951, v1, 641.

9. Best, C. H., and Campbell, J., *J. Physiol.*, 1936, v86, 190.

10. Levin, L., and Farber, R. K., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 758.

11. Friedman, S. M., Friedman, C. L., and Nakashima, M., *Am. J. Physiol.*, 1950, v163, 319.

12. Baker, B. L., Schairer, M. A., Ingle, D. J., and Li, C. H., *Anat. Rec.*, 1950, v106, 345.

13. Mahler, A., *J. Biol. Chem.*, 1926, v69, 653.

14. Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, v52, 191.

15. Youngburg, G. E., and Youngburg, M. V., *J. Lab. and Clin. Med.*, 1930, v16, 158.

16. Hawk, L. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, 12th Ed., The Blakiston Co. Ed., Philadelphia, p541.

17. Booker, W. M., Dent, F. M., and Hayes, R. L., *Fed. Proc.*, 1951, v10, 17.

18. Migeon, C. J., *et al.*, in preparation.

19. Zilversmit, D. B., Stern, T. N., and Overman, R. R., *Am. J. Physiol.*, 1951, v164, 31.

20. Snedecor, G. W., *Statistical Methods*, Iowa State College Press, Ames, Iowa, 1948, p82.

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## Oncolytic Effect of Viruses in Tissue Cultures.* (19695)

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When Levaditi and Nicolau(1) described the propagation of herpes virus in mouse epitheliomas they were seeking a substrate on which to propagate the virus. These authors and others(2-4) later demonstrated that when avian pest virus localized and propagated in mouse tumor tissue, it exerted therein a cytolytic effect. This initiated similar studies with other viral agents such as neurotropic

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vaccinia(5), rabies(6), vaccinia(7), and lymphogranuloma venereum(8), but these resulted at most in some vitiating effect on transplants of the parasitized tumors. On the other hand, Virus III seemed to enhance the malignancy of a transplantable tumor of rabbits(9). Recent *in vivo* studies have indicated that Russian Spring-Summer encephalitis (RSSE)(10,11), St. Louis encephalitis (SLE) (12), and Bunyamwera virus(13) would parasitize and destroy transplantable mouse sarcomas. An avian lymphoid tumor propagated *in vivo* was destroyed by RSSE, SLE, Japanese B and West Nile viruses(14). Egypt 101 virus destroyed human epidermoid carcinoma growing in x-irradiated rats and in tissue culture(15). It is obvious that one of the major hazards to the application of virus therapy for human cancer may be the pathogenic effect of some viruses on the patient. Many of the agents thus far found to be lethal to the tumors are unfortunately no less lethal to the host.

The oncolytic effect of viruses on experimental tumors of rodents can be demonstrated *in vitro*. The roller tube culture technic described by Cameron(16) involves the propagation of plasma-embedded tumor implants with ox serum ultrafiltrate† as the principal nutrient in the supernate. Two transplantable sarcomas have been studied: 1, a methylcholanthrene-induced anaplastic sarcoma of the rat;‡ and 2, a chemically-induced fibrosarcoma in the $C_3H$ mouse. Both killed their hosts at approximately 2 months after inoculation. Four surgically excised tumors of human origin were also studied for their response to viral infection.

*Methods.* The tumors were minced with scissors and 4 to 5 pieces (about 1.0 mm in diameter) were implanted in coagulating chicken plasma, to which ox serum ultrafiltrate (30%), Hanks solution with phenol red indicator (60%) and chick embryo extract (10%) were added in 1.0 cc quantity. One hundred units each of penicillin and of streptomycin were added to each tube. The nutrient fluids were replaced twice per week and otherwise were adjusted to optimal pH with 1.4% $NaHCO_3$ when needed. Thirty to 40 tubes were assembled with each tumor and to groups of 5 a different virus was added, so diluted that the 0.05 ml inoculum contained either 1,000 $LD_{50}$ or 1,000 hemagglutinating units of virus. The viruses were inoculated with the fresh implant or after the viable tumor had grown well (48-72 hours). All of the implants were examined under low power magnification during the next 7-14 days. Each experiment was repeated at least 3 times. The viruses used and their menstruums were as follows: Newcastle disease (NDV) (California strain), mumps (Enders strain), and influenza A in chick embryo chorio-allantoic fluid (CAF); and rabies (CVS strain), herpes simplex (MF strain), neurotropic influenza A, and St. Louis encephalitis (SLE) (Hubbard strain) in mouse CNS tissues. Lansing poliomyelitis virus (LCR) was used in cotton rat CNS. All of the virus preparations were clarified at 5,000 rpm for 20 minutes and were stored in sealed ampules in the $CO_2$ chest.

*Results.* The rat sarcoma implants rapidly metabolized the nutrient fluid causing the indicator to turn yellow within 24 hours. The untreated cells grew into the periphery as a solid mass of round cells. Herpes, influenza A (CAF) and LCR viruses exerted little effect on its growth properties. Rabies virus seemed to enhance the growth of this tumor tissue whereas SLE virus destroyed the implant and its outgrowth within 3 days (Fig. 1). The nutrient fluid for the SLE-treated tissues remained basic. When tumors were infected with SLE virus at implantation they grew poorly from the start and cytolysis was complete by the second and third days. When SLE virus was added 2 days after implantation it killed the tumor tissue within 48 hours. Neurotropic influenza A virus caused much deterioration of the implant and its outgrowth but only after a delay of 5-6 days. The cytolytic effect of the latter virus was not complete.

The fibro-sarcoma implants from $C_3H$ mice demonstrated fibroblastic outgrowths within 24-36 hours. By 48 hours each implant was surrounded by a grossly visible "halo" of

† Microbiological Associates, Bethesda, Md.

‡ Strain initiated by the M. D. Anderson Foundation for Cancer Research, Houston.

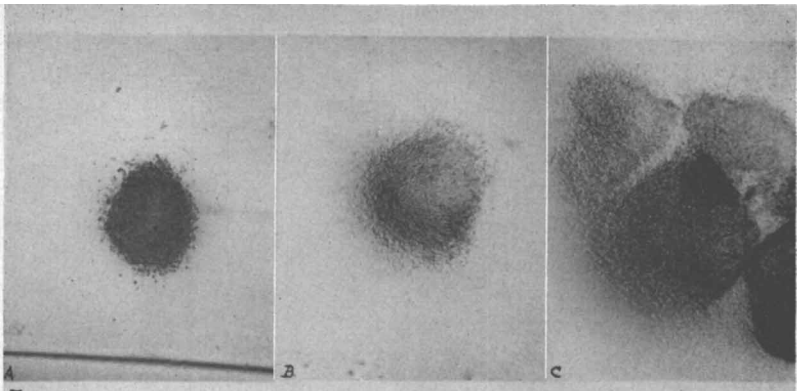


FIG. 1 RAT SARCOMA (ANDERSON) —48 HOURS

A +SLE Virus B No Virus C Rabies Virus

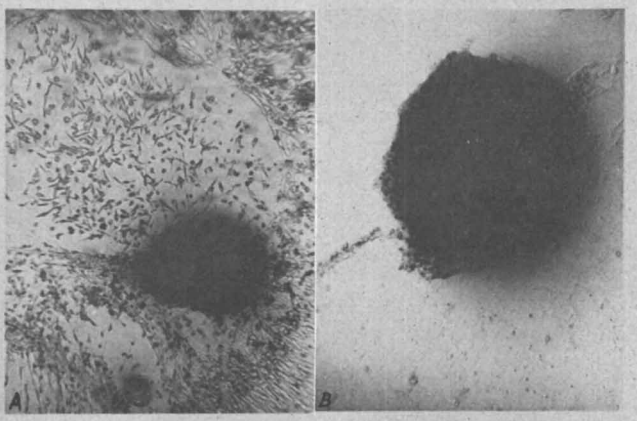


FIG. 2 MOUSE SARCOMA

A. Normal B. Effect of SLE Virus— 48 hours after inoculation

HUMAN MALIGNANT PAROTID TUMOR (Clon)

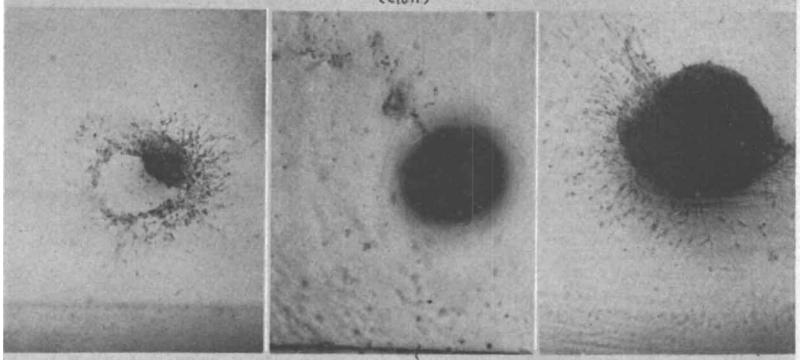

FIG. 3

10 days after adding NDV Virus | 8 days after adding SLE Virus | 10 days after adding Rabies Virus

cellular growth. Plasma liquification appeared in some areas of outgrowth causing the elongated cells to round up. Such tumor outgrowth often equalled the size of the implant within 72 hours. When NDV, rabies, influenza A (CAF), and LCR viruses were added to 48-hour-old implants there was no apparent change in the growth pattern. The rabies-infected tissues appeared to grow slightly more extensively. SLE virus caused fragmentation of the "halo" within 24 hours and death of all tissues by 48 hours (Fig. 2). Twenty-four hours after adding neurotropic influenza virus, the cellular outgrowths appeared granular and some degeneration of cells was evident at 48 hours. Most of the tissues infected by the latter virus were degenerated at 72 hours; however, this latter effect was not complete: the cells were shrunken, opaque and degenerating, but occasional healthy cells were observed throughout the following week.

Of the human tumors studied, a melanoma of low malignancy, which was excised from the leg of a 39-year-old white female, did not grow in tissue cultures. A scirrhous fibrosarcoma from the jaw of a 54-year-old white male also grew poorly in tissue cultures.

An undifferentiated parotid tumor§ from a 44-year-old white male grew well and implants were inoculated with virus on the fourth day. Those tissues to which NDV and rabies viruses were added continued to grow very well. SLE virus caused a visible shrinkage of the growth "halo" within 24 hours, which became granular and degenerated within 48 hours. By the sixth day the entire implant was disintegrated (Fig. 3). Neurotropic influenza A virus caused a similar sequence which appeared to be completely destructive by the eighth day.

A human anaplastic adenocarcinoma¶ within the abdominal cavity was collected with ascitic fluid. Implants thereof grew extensively over the wall of the tube and acidified the nutrient fluid within 24 hours. When virus was added after 24 hours of growth, NDV did not interfere with growth, rabies caused it to grow more extensively and SLE caused the cells to become pyknotic, crenated and many cells were disintegrated. On the fifth day following the addition of virus, the implants with rabies virus were growing profusely, while those with SLE were acellular.

*Discussion.* The tissue culture technic is a useful method for determining the virus susceptibility of a growing tumor. It is obvious that not all viruses exert the same cytopathogenic effect. There are some (NDV, LCR, influenza) without apparent influence, whereas another (rabies) may actually speed up the rate of growth of tumor tissue. This lack of effect has already been studied *in vivo* with influenza and herpes(17). The destruction of tissue was also reflected by the color of the indicator in the nutrient fluid. Actively growing tissues quickly acidified the medium, whereas such fluids remained basic when the tissues were killed. This color change has been found useful in studying the effect of poliomyelitis virus in tissue cultures(18).

Neurotropic influenza virus eventually destroyed the mouse fibro-sarcoma and interfered with growth of the rat sarcoma; however, we can postulate that the intact animal would have been immunized to this virus before the oncolytic process was complete. The oncolytic effect of neurotropic influenza virus contrasted sharply with the innocuous effect of chick embryo adapted virus of the same type. Perhaps a street strain of rabies virus might reverse the cell stimulating effect of the fixed rabies virus described herein. SLE virus appeared to kill the tumor implants within 48 hours after inoculation and would appear to be the agent of choice for all of the tumors (rodent and human) studied. Its pathogenic properties in the human, however, might cause one to feel that we would only be substituting one serious affliction with another if this virus was to be inoculated into a cancer patient.

If the antibody producing mechanism could be suppressed, perhaps a less lethal agent such as neurotropic influenza A virus might eventually destroy the tumor. Influenza is a rather common disease and it might be expected that the patient is already refractory to infection with such an agent, thereby obviating its effectiveness from the start. By incorporating the patient's serum into the nutrient fluid, the

§ Supplied by Dr. Truman Blocker, U. Texas Med. Branch, Galveston, Texas.

¶ Supplied by Dr. Paul Howe, M. D. Anderson Hospital, Houston, Texas.

immune status of the host may be determined simultaneously with the oncolytic effect of the virus.

The authors subscribe to the contention of Toolan and Moore(15) that a preliminary determination of viral sensitivity for each tumor should precede any consideration of therapy through viral infection. The tissue culture technic described provides a relatively rapid method of study by which a wide range of viruses can be studied for their effect on each human tumor. By this method, too, new viruses can be surveyed for possible cytopathogenic effect, with the hope that an agent with selective effect on the tumor can be either found or developed.

*Summary.* Tumor implants, growing in roller tube cultures, demonstrated a marked oncolytic response to St. Louis encephalitis infection. Two human and 2 rodent tumors were destroyed completely by SLE virus, less completely by neurotropic influenza virus. Infections with Lansing poliomyelitis, Newcastle disease, herpes and chick embryo adapted influenza A viruses did not interfere with growth of tissue implants. Rabies virus appeared to stimulate the growth of such tumor implants. The tissue culture technic can be used as a survey tool in rapidly determining the cytopathogenic effect of viral agents on tumors.

1. Levaditi, C., and Nicolau, S., *C. R. Soc. de Biol.,* 1922, v87, 498.
2. Levaditi, C., Schoen, R., and Reinie, L., *C. R. Soc. de Biol.,* 1937, v124, 711.
3. Levaditi, C., and Haber, P., *Revue d. immunol.,* 1937, v3, 5.
4. Haber, P., and Coquoin, M., *C. R. Soc. de Biol.,* 1937, v125, 238.
5. Turner, J. C., and Mulliken, B., *Cancer Research,* 1947, v7, 774.
6. Schoen, R., *C. R. Soc. de Biol.,* 1937, v125, 939.
7. Turner, J. C., Mulliken, B., and Kritzler, R. A., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 304.
8. Schoen, R., *C. R. Soc. de Biol.,* 1937, v125, 845.
9. Pearce, Louise, and Rivers, T. M., *J. Exp. Med.,* 1927, v46, 81.
10. Moore, Alice E., *Cancer,* 1949, v2, 525.
11. ———, *Cancer,* 1951, v4, 375.
12. Buckley, S. M., Buckley, J. J., and Snipes, A. E., *Cancer,* 1951, v4, 367.
13. Koprowska, I., and Koprowski, H., *Fed. Proc.,* 1952, v11, 420.
14. Sharples, G. R., Davies, M. C., and Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 270.
15. Toolan, Helene W., and Moore, Alice E., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 697.
16. Cameron, Gladys, *Tissue Culture Technique,* Academic Press Inc., New York, 1950, 1-191.
17. Moore, Alice E., *Cancer,* 1949, v2, 516.
18. Robbins, F. C., Enders, J. F., and Weller, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 370.

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## Effect of Antibiotics on Obstructive Appendicitis in Rabbits.* (19696)

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Previous work from this laboratory has shown that the cecal appendage of man, the chimpanzee and the rabbit possess a secretory capacity(1,2). When the orifice of the appendix is obstructed the "closed-loop syndrome" accompanied by perforation occurs. In animals in which a secretory capacity cannot be demonstrated, obstruction of the cecal appendage does not lead to perforation(3,4). Recently it has been shown that antibiotics reduce the mortality from appendicitis and its complications in man(5,6).

It seemed desirable to test the effects of antibiotics on the obstructed appendix in the rabbit to ascertain: 1) whether antibiotics reduce the mortality in this animal, and if so 2) whether they exert their effects through reduction in secretion or by other mechanisms.

*Experimental procedure.* Adult white rabbits of both sexes, weighing 1.8 to 4.3 kg were anesthetized with nembutal-ether and under surgical asepsis the appendicocecal juncture

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