Attempts to Extract Infective Nucleic Acid of Rous Sarcoma Virus.* (25947)

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Although infectivity of viral nucleic acid (NA) has been demonstrated for 2 plant viruses(1,2,3), several animal viruses(4-13) and T2 bacteriophage(14) the only oncogenic virus for which an infectious NA has been reported is polyoma virus. The original report(16) and a subsequent paper(17) indicate that infective polyoma NA is deoxyribose nucleic acid (DNA) while a recent report suggests that it may be ribose nucleic acid (RNA)(18). Failure to obtain an infective NA from Rous sarcoma virus (RSV) by cold phenol extraction was reported(19). The present study, which also reports failure to isolate infectious NA from RSV is presented because of the variety of technics used, because these negative results suggest that RSV might be fundamentally different from viruses which have yielded infectious NA, because in some experiments non-transmissible cytologic effects were observed which may be of biological significance, and because the studies cannot at this time be continued.

Methods. Two virus lines were used, both originally supplied by Dr. Ray Bryan. "M" line, maintained by both chick wing web (WW) and chick embryo chorioallantoic membrane (CAM) passage, titered about 10^{-5} in both CAM and WW. "B" line, maintained by Bryan's early passage technic(20) titered 10^{-7} or 10^{-8} by CAM or WW. Time of harvest was varied from day 3 to 10 for wing tumors and from day 2 to 7 for infected CAMs and the tissues were frozen immediately thereafter at −70°C (dry ice). Reagents were added directly to the frozen tissue in the homogenizer. The cold phenol technic(1) was used on one CAM and 3 WW preparations of M line virus, and one CAM stock of B line virus. Temperature was approximately 0 to 5°C (ice-water bath). Cadavarine 15 γ/ml was added to one cold phenol preparation to "stabilize" NA(21). Hot phenol technic(9) but with pH 6.7 0.14 M sodium citrate buffer was used on one WW and 9 CAM preparations of B line virus. Temperature was varied between 42° and 50°C. Proportion of the water-phenol interphase which was discarded was also varied. In 2 hot phenol experiments NA was precipitated with 67% ethanol or isopropanol. Sodium deoxycholate (SDC)(15) at final concentrations from 0.03% to 0.67% and with 37°C incubation for periods of 1 to 60 minutes was used in 10 experiments with a single RSV stock prepared from B-line infected CAMs. In 7 of these experiments the SDC was in solution for 2-7 days at 4°C prior to use, but in 3 it was used immediately after preparation. In 3 SDC experiments chymotrypsin 0.5% plus papain 0.5% were incubated with the tumor suspension at 4°C for 4 to 17 hours prior to SDC treatment to inactivate tissue ribonuclease. This did not inactivate virus but the effect on nucleases was not ascertained. In each experiment untreated tumor suspension, the NA preparation, and a ribonuclease-treated aliquot of the NA were tested simultaneously for infectivity by the same technics. Eggs and chickens were white leghorn, from Shamrock Farms, New Brunswick, N. J. NA preparations were tested as undiluted material equivalent to approximately 10% original tissue suspension, in groups of 25 to 40 eggs or chicks. Tissue culture (TC) tests were made in duplicate and usually included 1:5 and 1:25 dilutions as well as undiluted NA. In all but 3 of 25 experiments, CAMs of 9 or 12 day embryonated chicken eggs were inoculated with 0.2 ml of test materials and were examined for pocks on day 19 or 20. Titers on graphs indicate number of CAM-pock-forming units (PFU) per gram of tissue based on serial log dilutions in groups of 5 to 7 eggs. One of the cold-phenol preparations was inoculated

* Studies supported in part by Phoebe Waterman Fund, Nat. Cancer Inst., U.S.P.H.S., and Atomic Energy Comm.

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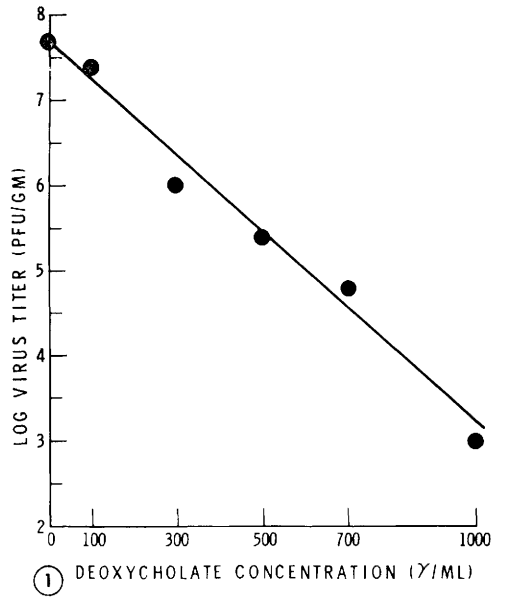
intravenously by Dr. Spencer Munroe into 12-day chick embryos (0.1 ml into veins of the CAM) and 1-day old chicks. Wing web inoculations were used in almost all phenol experiments and half of the SDC studies. Chicks were observed for 6-8 weeks. Hyaluronidase (Alidase, Searle Co.) was used with 2 of the hot phenol preparations at 3 units/ml as suggested by Prince(22). In one cold phenol experiment wings were injected with 0.2 ml of a 2% suspension of diatomaceous earth (Fuller earth powder, Mathewson Co.) 2 hours prior to RNA inoculation at same site, since a previous experiment had indicated that this procedure increased sensitivity of virus detection 10-fold. TCs were monolayers on glass. An agar overlay over chick embryo fibroblasts (23) was used for 10 of the 12 hot phenol preparations through the collaboration of Drs. Maxwell Eidinoff and Arnold Rich. Other TC studies used fluid medium consisting of Gey's salt solution, horse serum, and chick embryo extract. Medium from fluid cultures was routinely passed to CAM and/or WW 7 and 14 days after inoculation. Mengo(24), West Nile(25) and Semliki Forest(26) virus preparations were mouse brain suspensions harvested from paralyzed mice and stored on dry ice. Infectivity was titered by intracerebral (i.c.) inoculations of 16-18 g Swiss white mice (Taconic Farms) with 0.03 ml of serial 10-fold dilutions of NA extracts or brain suspensions and expressed as dilutions causing 50% mortality (LD_{50}).

Results. Cold phenol treatment of Mengo and Semliki Forest viruses and hot phenol treatment of West Nile virus (all as infected adult mouse brain preparations) yielded products with an ultraviolet absorption maximum at 255-260 and infectivity for mice which was abolished by incubation with 25 to 100 γ/ml RNAse for 5-20 minutes at 37°C. Intracerebral LD_{50} titers in 4-6-week-old Swiss White mice for initial virus stocks *vs* NA extracts were: Mengo $10^{-6.5}/10^{-2.5}$; Semliki Forest $10^{-7.2}/10^{-1.7}$; West Nile $>10^{-7.5}/10^{-1.8}$. These results confirm the more detailed studies of others(5,6,12) and indicate that these technics as used in this laboratory did yield an infective viral NA with suitable viruses.

Fifteen attempts to extract infectious nucleic acid from Rous sarcoma virus with cold or hot phenol technics using a variety of virus sources, chemical manipulations and detection systems yielded no tumors or other transmissible lesions. The only phenomenon suggestive of viral activity was the appearance of foci of rounded cells in chick fibroblast cultures(23) with 3 of the 7 hot phenol preparations so studied. These foci resembled those in the whole-virus controls. RNAse treated NA did not yield such lesions. This effect could not be transmitted by passage of tissue cultures (cells scraped from glass and emulsified) into new tissue cultures, eggs, or chicks. In contrast, tissue cultures inoculated with whole RSV so diluted as to yield only 2 foci/plate were passed without difficulty by all these technics. Thus the observed lesions seem to be the result of RNA from the Rous virus preparation (B-line on CAM, titer 10^{-7}), but there is no evidence that they were due to or accompanied by virus propagation. In these 3 experiments NA was tested undiluted and at 1:5 and 1:25 dilutions. At 1:25 numbers of foci per gram of original tissue were 500, 200 and 2 respectively. These numbers were as great or greater than at the lower dilutions, suggesting an inhibitor in the NA preparation.

SDC inactivated RSV rapidly and in direct relation to SDC concentration (Fig. 1). With 0.03% SDC at 0°C a 2-log (99%) inactivation occurred within 1 minute, but thereafter inactivation proceeded much more slowly (Fig. 2). Failure of RNAse to alter this inactivation curve eliminated the possibility that the residual infectivity might be due to RNA. The prompt drop when more SDC was added indicates that leveling-off of inactivation was due to disappearance of effective concentrations of SDC. Much of the SDC was probably inactivated by non-viral constituents of the RSV suspension, since it was shown to be inactivated by prior admixture with bovine serum albumen.

Incubation of one hot-phenol-extracted RSV preparation with an untreated RSV preparation or with RSV partially inactivated by UV irradiation did not affect infectivity titers. Incubation of virus preparations with



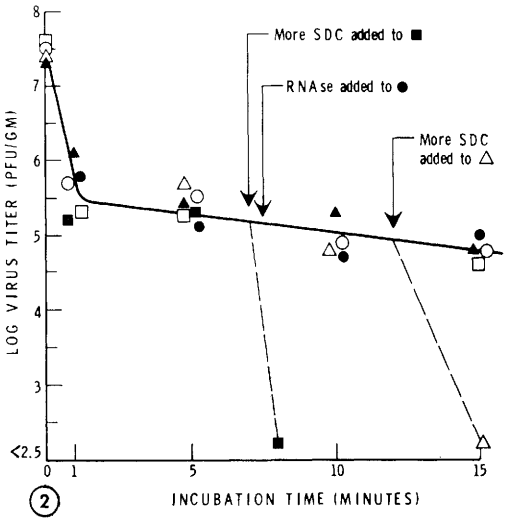


FIG. 1. Decreasing infectivity of RSV in increasing concentrations of SDC at 0-2° (ice water bath) for 5 min.

FIG. 2. Partial inactivation of RSV infectivity by 0.03% SDC at 0-4°C. Infectivity of partially inactivated virus was unaffected by RNase, but abolished by added SDC.

DNAse prior to hot phenol extraction failed to yield an infective NA. These experiments failed to support the working hypotheses that NA preparations might contain inhibitors or incomplete viral components capable of restricted viral activity.

Summary. Sodium deoxycholate and hot and cold phenol were used to extract nucleic acid from Rous sarcoma. The hot phenol technic in some experiments yielded a RNAse-sensitive product that caused non-transmissible focal lesions in chick fibroblast tissue cultures, but no extracts initiated a transmissible infection in tissue cultures, chicks or embryonated eggs. The same hot and cold phenol technics did yield infective nucleic acid from West Nile, Mengo, and Semliki Forest virus preparations. Rous sarcoma virus was rapidly inactivated by deoxycholate.

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Received June 20, 1960. P.S.E.B.M., 1960, v104.

Effect of Triparanol on Beta-Aminopropionitrile-Induced Dissecting Aneurysm and Blood Lipid Levels in the Turkey.* (25948)

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A probable cause of beta-aminopropionitrile (BAPN)-induced dissecting aneurysm in turkeys appears to involve defective connective tissue metabolism, since it has been shown(1, 2) that this is a known effect of the agent. However, the presence of fatty degeneration in the intimal layer of the aorta in the region of rupture in naturally occurring cases(3) of internal bleeding and the gross observation of yellow fatty areas in the lumen of the aorta in the area of rupture in BAPN-treated birds (4,5), raised the question of the importance of atherosclerotic lesions in development of the malady. The object of this study was to determine whether triparanol,† a drug having the property of lowering the ratio of blood cholesterol to phospholipid(6), could reduce the severity of BAPN-induced dissecting aneurysm in turkeys. The effects of triparanol on growth rate and plasma cholesterol and phospholipid levels are also reported here.

Methods. Turkey poults were housed during the first 3 weeks in electrically heated batteries with raised wire-mesh floors, then were moved to conventional growing batteries. Details showing breed, number of birds per group, and experimental duration are given in the tables. The diet is presented in Table I. Triparanol and beta-aminopropionitrile fumarate were added as one per cent premixes in ground corn. Vitamin mixture C-5 supplied the following per 5 g of mixture: biotin 0.2 mg, menadione sodium bisulfite—63% USP 2 mg, pyridoxine · HCl 2 mg, folic acid 2 mg, thiamine · HCl 2 mg, riboflavin 5 mg, calcium pantothenate 10 mg, niacin 40 mg, Vit. B_{12}—0.1% triturate in mannitol 30 mg, Cerelose 200 mg, and corn meal to 5 g. All birds succumbing during an experiment and many surviving birds were subjected to necropsy examination. Detailed pathological findings will be published later. Blood samples were drawn in a heparinized syringe as indicated in the tables. Plasma cholesterol and phospholipid were determined by the methods of Abell *et al.*(8) and Zilversmit and Davis(9), respectively.

Results. The depressing action of triparanol on turkey growth is shown in Fig. 1. The depression appears to be logarithmic in nature. The intercept of this straight line relationship with control growth was plotted at 10.6 ppm and 3.9 ppm triparanol for turkeys 5 and 3 weeks on experiment, respectively. It is felt that this is the approximate dosage range at which triparanol begins to depress growth rate in turkeys.

Triparanol did not protect against BAPN-induced aortic rupture (Table II). The hemorrhaging index, as defined by Waibel and Pomeroy(5), was at least as great when triparanol was added to the 0.05% level of

* Published as paper no. 4343, Scientific Journal Series at Minn. Agri. Expt. Station This investigation was supported in part by research grant from Nat. Heart Inst., P.H.S.

† Triparanol was formerly designated by code number MER-29. It is produced by Merrell Co., Cincinnati, O. Formula is 1-[(4-diethylaminoethoxy) phenyl]-1-(p-tolyl)-2-(p-chlorophenyl) ethanol.