collagen, content of epiphysial cartilage from rats treated with AAN is not reduced from the normal. Homogenized fractions of cartilage from rats which have received AAN are virtually devoid of collagen fibrils when examined with electron microscope. The defect appears to be a failure of the tropocollagen molecule to form collagen fibers.

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Cytopathogenicity in Tissue Cultures by a Tumor Virus from Mice. (24205)

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A virus, which we shall refer to as SE polyoma virus, was recovered from tissue cultures inoculated with tumor material from mice and was shown to induce multiple tumors in mice(1,2,3) and hamsters(4). The present report describes cytopathic effects which appear to be produced by the SE polyoma virus in tissue cultures. The results so far obtained show that these effects parallel the capacity of tissue culture fluids to induce tumors in hamsters.

Methods. Tissue cultures from embryos of Swiss mice were propagated in 2 ounce prescription bottles by modification of the trypsin digest method of Youngner(5). Synthetic medium 199(6) containing 2% inactivated calf serum was used to initiate growth, and medium 199 with 1% inactivated rabbit serum was used as maintenance medium. For quantitative titration or serum-virus neutralization tests the cells were retrypsinized with 0.25% trypsin in Earle's balanced salt solution(7) at 35°C for 15 to 20 minutes, centrifuged, washed, resuspended in initial medium and dispensed in roller tubes. The cells in the retrypsinized cultures were clear and uniform. The strain of the agent used, originated from liver and spleen of a single AKR mouse with spontaneous leukemia and was passed serially into 3 newborn mice, with intervening passages in tissue cultures, followed by 2 to 4 passages in newborn hamsters with intervening tissue culture passages. Each passage animal had developed neoplasms. Tumor tissue from only one animal from each litter was used for passage. Titration of virus at several passage numbers was made in roller tube cultures of retrypsinized mouse embryo cells by preparing decimal dilutions and inoculating 4 tubes/dilution with 0.5 ml each. The cultures were refed at weekly intervals with 2 ml of nutrient fluid. Serum-virus neutralization tests were also carried out in roller tube cultures. Mixtures of equal volumes of 10-fold dilutions of the polyoma virus and 1% antipolyoma virus rabbit serum and mixtures of similar dilutions of virus and normal rabbit serum, were held at 36°C for 4 hours before being added to the roller tubes. The antiserum was made by hyperimmunizing rabbits with fluids from tissue cultures infected with the virus. The tubes were refed after one week with medium containing 1% immune and normal rabbit serum respectively. The final microscopic examinations were made at 3 weeks. To demonstrate induction of tumors, hamsters 1 to 3 days of age were injected with 0.2 ml doses of tissue culture fluid

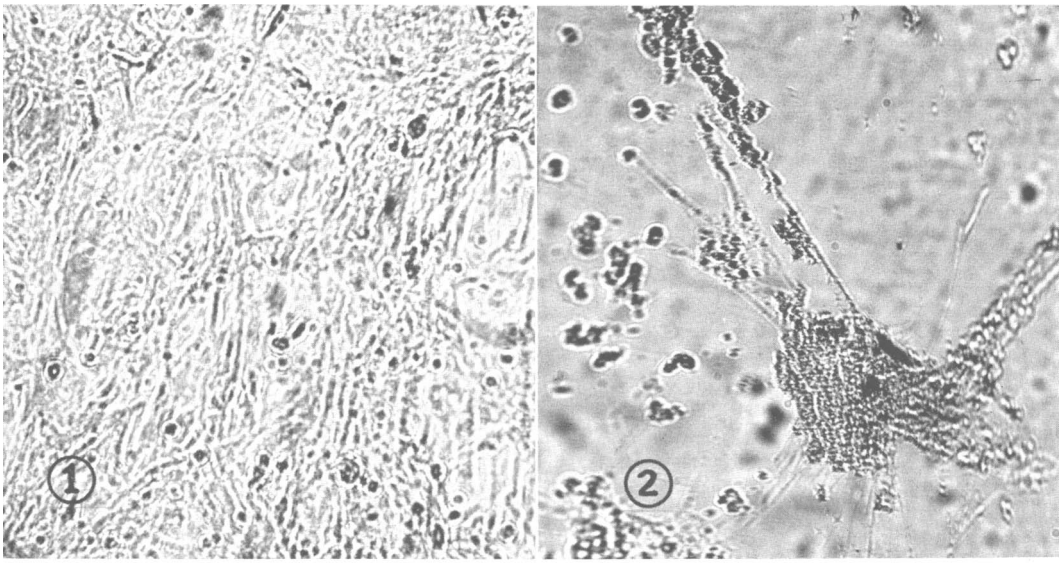

FIG. 1. 19-day-old culture of mouse embryo cells. Unstained. × 60.
FIG. 2. Cytopathic changes in mouse embryo cells 14 days after inoculation with SE polyoma virus. Unstained. × 60.

subcutaneously. The young were divided among mothers as equally as possible so that each set of hamsters inoculated were the same age.

Results. During many early tissue culture passages of the virus, infected cultures did not appear to be different from the uninfected control cultures; in others slight cytopathogenic changes occurred in infected cultures at irregular intervals in 2- to 3-week-old cultures. As tissue culture or animal passages were continued these cytopathogenic changes in tissue cultures occurred regularly and degeneration was more extensive.

The changes consisted of patches of small dark cells which appeared on sheets of normal appearing cells after 4 to 7 days. The number of these pyknotic cells continued to progress in 1 to 2 weeks until the majority of cells were effected, causing many of them to fall from the glass (Figs. 1 and 2). Subpassages of fluids from such cultures regularly produced cytopathic changes in other mouse embryo cultures.

Titrations showed a TID^{50} of approximately 10^{-3} at 2 weeks and 10^{-4} or 10^{-6} at 3 weeks. The endpoints were more distinct at 3 weeks.

Reduction of the titer of the virus was noted when cultures were inoculated with mixtures of the virus and 1% homologous antiserum against the polyoma virus, as compared to similar cultures inoculated with mixtures of virus and normal rabbit serum. Degenerative changes were noted in cultures with undiluted and 10^{-1} virus combined with immune rabbit serum but not at higher dilutions. Titration of the virus with normal rabbit serum showed cytopathic changes through the 10^{-5} dilution. Immune rabbit serum was effective in preventing induction of tumors when combined with virus before administration to newborn mice(8).

Infectivity for hamsters appeared to be correlated with cytopathogenic changes in tissue cultures. To demonstrate this, infected fluids from tissue cultures, which showed cytopathogenic changes as a result of being incubated with a 10^{-5} dilution of virus and fluid from cultures incubated with a 10^{-6} dilution of virus which did not cause degenerative changes, were injected into hamsters. The results of a single experiment are given in Table I. All hamsters inoculated with fluid from cultures that failed to show degenerative changes were free of tumors until the 34th day, and 6 of the 11 were free of tumors on the 46th day. Two hamsters were missing on the 34th day.

TABLE I. Effect of Inoculating Hamsters with Supernatant Fluid from Tissue Cultures Which Showed Cytopathic Changes, and with Fluid from Cultures Which Did Not Show Such Changes.

Virus in tissue cultures			No. of hamsters					
			At 26 days			At 43 days		
Inoculum	Cytopathogenicity	Inoculated	Lost	With tumors	Surviving and free of tumors	Lost	With tumors	Surviving and free of tumors
10^{-5}	+	12	7	5	0	0	0	0
10^{-6}	0	11	0	0	11	2	3	6

TABLE II. Results of Infecting Hamsters with SE Polyoma Virus Filtered through Gradocol Membranes and with Supernatant Fluids of Tissue Cultures Incubated with the Filtrates.

Pore size of filter, mμ	No. of passages of filtrates in tissue culture	Cytopathogenicity	No. of hamsters			
			Inj.	Cannibalized	With tumors	Surviving and free of tumors
Unfiltered	none		8	3	3	2
220	"		8	0	1	7
120	"		10	0	3	7
"	2	+	9	1	8	0
"	3	+	13	9	4	0
77	none		9	0	0	9
"	4	0	12	0	0	12
43	none		11	0	0	11
"	4	0	26	0	0	26

On the other hand all 12 animals inoculated with fluid from cultures which showed cytopathic changes, had tumors or were dead 26 days after injection. Seven were cannibalized on 17th to 21st day and were not examined.

Evidence of correlation of cytopathogenicity and tumor induction was also obtained from ultrafiltration experiments designed to establish size of the infective agent.

Virus-infected fluids were filtered through a Seitz S3 filter and a gradocol membrane of 590 mμ porosity. Portions of this filtrate were then filtered through 4 membranes of different pore size (Table II). Fluid passed through membranes of 220 and 120 mμ average pore size caused cytopathic changes in tissue cultures and hamsters inoculated with these filtrates or with filtrates after passage in tissue cultures developed tumors.

The fluid passed through membranes of 77 and 43 mμ porosity did not cause cellular changes in tissue cultures. These filtrates or the fluid from tissue cultures inoculated with these filtrates, failed to induce tumors in hamsters after 128 days.

The results thus far are entirely compatible with the hypothesis that the tumor virus causes cytopathic changes in mouse embryo tissue culture. Because of the possibility of the presence of a concomitant virus in any tissue culture system, further experiments on correlation of tumor induction and cytopathic changes in tissue cultures are in progress. The practical importance of a tool to determine capacity of a fluid to induce tumors is obvious and prompts this early report.

Summary. Cytopathogenic changes were observed in mouse embryo cell cultures that had been inoculated with tissue culture-grown virus isolated from mouse tumor tissues. Quantitative assays of the virus, referred to as the SE polyoma virus, and serum-virus neutralization tests were correlated with the induction of tumors in hamsters.

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Effect of Major Dietary Constituents on Growth Response of Rats to Vit B_{12}.* (24206)

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Hartman *et al.*(1) reported that high protein rations produced a deleterious and sometimes fatal effect in Vit. B_{12} deficient rats. Bosshardt *et al.*(2) found that rations high in fat had a sparing action in mice upon the requirement for Vit. B_{12}. McCollum and Chow(3) reported that casein rations high in carbohydrate increased the Vit. B_{12} deficiency in female rats. The purpose of this study was to determine which of the 3 basic dietary constituents—fats, carbohydrates, and proteins—would produce the greatest growth response to Vit. B_{12} when plant protein rations were used.

Method. Adult female Sprague-Dawley rats (100 days old) were placed on a corn-soybean ration(4) at mating and maintained on this ration during pregnancy and lactation. The young had access to this ration until they were placed on experimental rations at weights of 40 ± 5 g. The care and handling of animals have been described previously(4). The rations (Table I) were adjusted so that the same percentage of calories was derived from protein in the high carbohydrate and high fat rations. Protein was isocaloric in the high protein and high protein-high fat rations. All animals received, in addition, 2 drops of Halver Oil weekly.

Results. The results are tabulated in Tables II and III. The differences in growth between the supplemented and unsupplemented rats on the high carbohydrate and high fat rations were not great. Similar results were also obtained in rats on the low protein diet in Series I. Growth differences obtained between supplemented and deficient animals fed the high protein and high protein-high fat diets were in every instance highly significant ($P<0.01$). Large variations in growth occurred with these diets with the greatest variation in the non-supplemented groups.

TABLE I. Composition of Rations.

Ingredients	High carbohydrate	High fat	High protein, high fat	High protein	Low protein
Drackett protein*	24.	35.	66.5	54.	16.2
Corn starch	62.5	16.5		32.5	70.5
Hydrogenated fat†		35.	20.		
Corn oil	5.	5.	5.	5.	5.
Salts XIV‡	4.	4.	4.	4.	4.
Vit. mix§	2.	2.	2.	2.	2.
Alphacel‡	2.	2.	2.	2.	2.
Methionine	.5	.5	.5	.5	.5
Vit. B_{12} mix‖ when indicated	.1	.1	.1	.1	.1

* Drackett Assay Protein G-1 81% protein (N × 6.25). † Crisco. ‡ Nutritional Biochemicals. § *J. Biol. Chem.*, 1954, v206, 765. ‖ 5% 0.001 Triturate B_{12} (Merck), 95% sucrose.

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