not significant (p greater than 20%) and week-to-week changes are very small(1,5,6). According to these reports and our own experience, we cannot consider the changes observed in our study as normal variations in serum copper level, as all individuals showed definite increases. Since both androgen and estrogen increased serum copper content in both male and female, there must exist some relationship between sex hormone level in the body and serum copper content.

Summary. 1. Administration of testosterone propionate or estradiol benzoate to healthy elderly males and post-menopausal women increased significantly their serum copper content. 2. Eight weeks after discontinuance of androgen or estrogen administration, serum copper values dropped back to, or very close to, preadministration levels.

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Transplantation of Polyoma Virus Induced Tumor in the Hamster. (25156)

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Polyoma virus was isolated from cell-free extracts of parotid gland tumors which had been produced by inoculation of newborn mice with similar extracts from leukemia #60 or spontaneous AKR leukemias(1). The demonstration that this agent would multiply in monkey kidney tissue culture(2), cause cytopathic lesions in mouse embryo tissue culture(3), and hemagglutination of guinea pig rbc(4) have made experiments with this virus practical. The virus is capable of producing a variety of tumor types when inoculated into newborn mice(5) and hamsters(3) but no evidence of tumor production or infection in adults except production of antiviral antibodies. This is a report of the successful transplantation in adult Syrian hamsters of a sarcoma produced by inoculation of a newborn hamster with polyoma virus, cultivation of the transplantable tumor in tissue culture and the relationship of virus and viral antibody to its growth.

Methods. (a) *Transplantation.* Healthy appearing areas of tumors were removed and minced with scissors in Eagle's tissue culture medium to give a heavy suspension of tumor fragments and 0.2 ml inoculated subcutaneously into the right scapular area of either newborn or adult hamsters. Animals were observed twice weekly for signs of tumor development. (b) *Demonstration of virus.* Either mouse embryo tissue cultures were inoculated and observed for cytopathic effect and development of hemagglutinins (HA) or 5 adult mice were inoculated intraperitoneally, bled 3 weeks later and their serum tested for hemagglutinin inhibiting antibodies(HI). (c) *HA and HI tests.* The technic and criteria described by Eddy, *et al.*(4) were used. (d) *Tissue culture.* Standard procedures of trypsinization and planting in T30 flasks were used with 10% calf serum Eagle's medium for growth and 2% horse serum Eagle's for maintenance. Maintenance medium was changed twice weekly.

Results. (a) *Transplantation series.* Hamster C474, kindly supplied by Dr. Bernice Eddy, had a subcutaneous sarcoma of approximately 5 weeks' duration, measuring 3 x 6 inches. Its serum had an HI titer of 640,

TABLE I. Transplantation History of C474 Tumor and Hemagglutination Inhibition (HI) Antibody Levels in Adult Hamsters.

Transfer #	Hamster #	Day: Palpable	Day: Transfer	HI antibodies: Day	HI antibodies: Titer*
1	2099	41	54		
2	1957	10	17	17	40
	1962	10		28	<40
	Litter	10	15	28	40
3	25	11	14	0	<40
				14	"
	8	11		0	40
				35	<40
	2041†	12		16	160
4	2030	10	20	0	<40
				20	"
	2027	10		0	<20
				20	"
				41	160
5	2031	8	20	20	<40
	2035	8			
6	1904	7	26	0	<40
				26	"
	1908	7		36	20
7	1901	7	16	16	<20
	1915	7		29	"
8	2210	6			
	2215	6			

* Reciprocal of highest dilution giving inhibition.

† Received transfer from P_2 suckling tumor.

This hamster had been inoculated at 5 days of age with mouse embryo tissue culture propagated polyoma virus and developed a subcutaneous tumor 7½ months later. A minced suspension of a portion of this tumor was inoculated IP into adult hamster 2099 with development of a subcutaneous tumor at site of inoculation 41 days later. This tumor has been transplanted 8 times (Table I). Another adult hamster (2098) also received a suspension of cells from original tumor by IP route and subsequently inoculated with frozen tumor suspension twice weekly for 8 doses. A large intraperitoneal tumor was first noticed in this hamster 84 days from first inoculation at which time its serum had an HI titer of 40. A portion of the original C474 tumor was emulsified, filtered through an ultra-fine sintered glass filter and inoculated subcutaneously into a litter of newborn hamsters. No tumors appeared in 90 days at which time their sera had a HI titer of <20. At time of second transfer the cell suspension was inoculated into newborn hamsters as well as into adults. Tumors appeared at the same time in different aged animals and both were successfully transferred to adult animals. In none of the transplant passages were metastases seen, although adjacent satellite tumors appeared in several hamsters after the primary was well developed. Once the tumors had grown beyond 2 inches in diameter, necrosis with hemorrhage usually ensued and this was the cause of death.

Included in Table I are the HI titers on animals used in the transplantation series. With but 2 exceptions, Transfer 3 and 4, the animals developing tumors from the transplants were negative for HI antibodies even at times when tumors were quite large.

Histological examination of the original and transplant passage #8 tumors was made by Dr. Samuel S. Spicer who described both as similar and either fibroma or fibrosarcoma in character.

(b) *Lack of immunity to transplanted tumors.* Minced tumor suspension from third transplant passage was inoculated into animals which received multiple doses of original C474 tumor, blood or skin; hamsters with a first, second or third transfer tumor; 2 hamsters hyperimmunized with polyoma virus; a hamster with a virus-induced tumor and normal adult hamsters. The results in Table II show that tumors appeared at 10 days in all animals and there was no marked difference in their growth. Table III shows results of another experiment in which an adult hamster previously hyperimmunized with polyoma virus and 2 normal adults received a suspension of transplant #6 tumor with some evidence that tumor growth in the immune animal was slower than in controls. Again, in those animals developing tumors, HI antibodies did not appear within 21 days after challenge at which time their transplanted tumors were quite large, except in those that already had antibodies before challenge.

(c) *Attempts to transplant hamster tumor in other species.* Tumor of transfer #3 was inoculated subcutaneously into the following adult animals: 2 white rats (0.2 ml), 2 guinea pigs (0.2 ml), 5 Swiss mice (0.05 ml),

TABLE II. Results of Challenge with Transfer #3 Tumor for Possible Immunity in Hamsters.

History before challenge	Size of tumor (inches) 10 days	28 days	HI antibody titer 0 day	21 days
6 doses whole blood from C474	1/8 × 1/4	1 × 1	ND	< 40
Idem	1/2 × 3/4	2 × 2	"	40
8 doses skin emulsion from C474	1/4 × 3/4	2 × 4	"	"
8 doses tumor C474; large IP tumor	1/4 × 1/2	Dead	40	< 40
1 dose polyoma virus 2 mo prior	1/2 × 3/4	2 1/2 × 1 1/2	>2560	>2560
Idem	1/4 × 1/2	2 × 2 1/2	"	"
P2 transfer of tumor cell suspension 1 mo before; large tumor of 3 wk duration	1/2 × 1	2 × 3	< 40	40
3 doses polyoma virus during preceding month	1/4 × 1/2	1 1/2 × 1 1/2	>2560	>2560
Idem	1/8 × 3/8	1 × 1 1/2	"	"
P3 transfer of tumor cells 3 wk before; tumor of 4 days' duration	1/8 × 1/4	2 × 2	160	80
Inoculated with virus as newborn 3 mo before; large subcut. tumor	1/2 × 1	2 × 3	40	40
Normal	1/2 × 1	Killed 20 days	< 40	< 40
"	1/2 × 3/4	2 × 2	< 20	*< 20

* On 41st day HI 160.

and intracutaneously into 1 monkey (0.05 ml) as well as into 2 hamsters (0.2 ml). No evidence of tumor developed in rats, guinea pigs or monkey in 90 days' observation and no HI antibodies developed in the first 21 days, whereas (Table I) Hamsters 2030 and 2027 developed tumors in 10 days. In all 5 adult mice, millet seed nodules could be palpated at site of inoculation by 10 days but these never progressed except in one mouse where by 26 days a mass approximately 7 mm in diameter had developed. All mice were negative for HI antibodies at 3 weeks. At 26 days, the mass in the 1 mouse was removed and trypsinized and the resultant single cell suspension inoculated into 5 adult mice and 2 adult hamsters. In 60 days no tumors appeared in the mice but the hamsters developed progressively growing tumors starting in 12 days. At 21 days 1 of these tumors was removed, minced and inoculated into 5 adult mice which remained normal for 40 days. The original mouse tumor was also grown in tissue culture where the cells were definitely smaller than in tissue cultures of hamster tumors. This tissue culture was tested for virus by mouse inoculation test over the 42 days of its maintenance with negative results. It is of interest that of 2 hamsters developing tumors on transfer of the mouse tumor cells, one killed at 21 days had a negative HI test but the other at 40 days had a titer of 160.

(d) *Tissue culture of transplanted tumor.* Tumor from hamster 2030 representing transfer #4 was trypsinized and established in tissue culture as were also the tumors produced in newborn hamsters after inoculation with transfer #1 cells. When the cells in these tissue cultures had grown out they were trypsinized and divided into 2 flasks for subculture. One of each subculture level was maintained without further passage. The cultures were held for 48 days (4 subcultures) and 55 days (5 subcultures) from their inception and supernates at the time of biweekly medium changes were tested for the presence of virus

TABLE III. Effect of Polyoma Virus Immunity on Progression of Transplanted Tumor in Hamsters.

History before challenge*	Size of tumor (inches) 10 day	16 day	29 day	HI antibody titer 0 day	Day after challenge
Normal	1/2 × 1/2	1 × 1		< 20	16 – <20
"	1/2 × 1	1 × 2	3 × 4	"	29 – "
Hyperimmunized with virus	1/4 × 1/4	1/4 × 1/2	1 × 1 1/2	>2560	>2560

* Challenged with tumor cell suspension of transplant passage #6.

by inoculation into mouse embryo cultures or into mice with completely negative results. When they were respectively 40, 30 and 23 days old, passage 2, 3 and 4 subcultures of 2030 tumor were trypsinized, pooled, and a suspension of cells inoculated into an adult hamster in which a rapidly progressing tumor appeared at 14 days. This hamster's serum had an HI titer of <20 at 27 days after receiving the cell inoculum.

Discussion. Ability of this polyoma virus induced fibrosarcoma to be transplanted into adult hamsters through 8 transplants establishes one additional criterion concerning its malignancy. The infrequency of development of anti-polyoma virus antibodies in those animals supporting growth of large transplanted tumors and the fact that presence of high antibody levels against the virus did not prevent a take of transplanted cells, raise questions concerning relationships between virus and tumor. Others have found that it is difficult to isolate the virus from tumors developing after infection of newborn animals but this has been explained as due to presence of high levels of antibody in serum and tumor at time of its emulsification. However, this cannot be the explanation in animals carrying transplanted tumors since no antibody was demonstrable at the time the tumor was harvested. Furthermore, on carrying the transplanted tumor through 5 tissue culture transfers over 55 days, no evidence of virus could be demonstrated. Yet virus was present in the original tumor since it was isolated from the second and third tissue culture transfer of that tumor. The late development of antibody in a few hamsters in the transplantation series suggests that virus is present in the tumors but is released very slowly and in amounts insufficient to act as an effective antigenic stimulus to the animal's immunological system.

Summary. A fibrosarcoma produced by inoculation of polyoma virus into a suckling hamster has been transplanted through 8 transfers in adult hamsters with preservation of its original histological character. In only an occasional animal supporting a transplanted tumor have anti-polyoma virus antibodies appeared. The transplanted tumor was carried through 5 tissue culture transfers without demonstration of virus release and tissue culture cells were capable of producing tumors in an adult hamster. After 26 days sojourn in an adult mouse, the transplanted hamster tumor still produced tumors in the hamster but was not transplantable in series in the mouse. The presence of tumors or high level of virus antibodies did not prevent a positive take with the transplantable tumor.

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Adenylic Acid and Adenosine Deaminase Activities in Rat Livers During Azo-Dye Carcinogenesis.* (25157)

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Adenylic acid and adenosine deaminases are 2 purine-metabolizing enzymes. The former catalyzes conversion of 5′-adenylic acid to 5′-inosinic acid while the latter catalyzes conversion of adenosine to inosine(1). Recently, it was shown that adenylic acid de-

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