note that the mean decrease in the activity of the G-6-PD of the red blood cell(13 and 7.3%) observed in our experiment is of an order of magnitude similar to the one expected using the calculation described above.

Obviously the changes observed are still within the normal range and any specific interpretation of the biological significance of these changes should be approached with caution. Nevertheless, it seems possible that *in vivo*, DHA or one of its metabolic products may function as a factor in regulating the activity of the enzyme glucose-6-phosphate dehydrogenase in red blood cells. If this also occurs in other tissues as well, DHA could play a very important role in influencing the rate of oxidation of glucose *via* the hexosemonophosphate shunt. The possible importance of this regulation in obesity has been pointed out by us(13) as well as others(14).

Summary. Dehydroepiandrosterone, a potent *in vitro* inhibitor of the enzyme glucose-6-phosphate dehydrogenase, produced a significant decrease in the activity of the glucose-6-phosphate dehydrogenase of the red blood cells of a normal adult man when administered orally in doses of 100 mg for 3 consecutive days.

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Tumor Induction in Thymectomized Rats by Murine Sarcoma Virus (Moloney) and Properties of the Induced Virus-free Tumor Cells. (32568)

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A murine sarcoma virus (MSV) isolated by Moloney from BALB/c mice inoculated with Moloney leukemogenic virus (MLV) induces tumors rapidly in most strains of newborn mice(1), but rarely induces tumors in intact newborn rats. Rat embryo cells in culture can be transformed by MSV and these transformed cells will induce tumor growth in young syngeneic hosts(2). Tumors have also been induced in Osborne-Mendel and Sprague-Dawley rats when injected intracerebrally at 3 days of age with MSV(3). It is known that thymectomy can influence the frequency of neoplasms induced by several tumor viruses (5,6). Thymectomized rats were therefore used to test their susceptibility to MSV. The biological properties of one of the rat tumors which does not produce infectious virus will be discussed here in detail.

Materials and methods. Rats. Inbred rat strains BN, Lewis and an Fl hybrid BN x Lewis obtained from Microbiological Associates, Walkersville, Md. were used.

Viruses. MSV, a tumor extract of BALB/c mice was supplied by Dr. Moloney of this institute. It was then grown on NIH Swiss mouse embryo cells. Tissue culture grown MLV was obtained from Dr. Hartley of the National Institute of Allergy and Infectious Disease and Rauscher leukemia virus (RV) from leukemic rat kidney cells in culture was supplied by Dr. Duc-Nguyen of this institute.

Media. Dulbecco Modified Eagle's Medium with 10% bovine fetal serum and 10% tryptose phosphate broth was used in all experiments.

Thymectomy. Rats were thymectomized within 24 hours after birth. In each experiment intact or sham operated litter mates were included. Thymectomized animals found to have residual thymus were excluded from the results.

MSV assay. MSV was assayed on mouse embryo cells by the method of Hartley and Rowe(7), and the titer was expressed as focus-forming units (FFU).

Marker rescue. Bottle cultures (1 oz. size) were seeded with 2×10^5 MSB-1 cells (see text for description) alone or 1×10^5 MSB-1 cells and 1×10^5 MEC in 4 ml media. Cultures were washed and infected with 0.2 ml leukemogenic viruses 24 hours later. Supernatants were harvested the day of transferring the cultures and were stored at −70°C until they were tested for focus-forming activity of virus on mouse embryo cells.

Results. Inbred rat strains, Lewis, BN and their F1 progenies were thymectomized within 24 hours after birth and were injected intramuscularly with MSV. Results (Table I) showed that 30-50% of thymectomized rats developed tumors at the site of inoculation with a latent period of 3-4 weeks and only 1 of their intact littermates developed a tumor.

Tumors induced in mice are not readily transplantable except occasionally in thymectomized and X-rayed recipients(8). In contrast tumors induced in rats can be readily transplanted to syngeneic hosts. To date we have established 2 transplantable tumors in Lewis rats and 3 in BN rats.

The 3rd transplant passage of one of the BN tumors, designated here as MSB-1, a neoplasm of epithilial origin, was trypsinized and was grown in tissue culture in Dulbecco modified Eagle's medium with 10% tryptose phosphate broth and 10% bovine fetal serum. After 2 passages in culture, cells were cloned on X-rayed (5000 r) rat feeder layer. A single clone was picked and grown into a culture. The culture consists of epithelial-like cells and cells tend to come off the surface when the culture ages. 5×10^3 cells are sufficient to induce tumor growth in 50% of adult BN rats. MSB-1 cells are apparently free of MLV as well as MSV for various attempts to demonstrate leukemia or sarcoma virus were unsuccessful. Cell-free culture fluid did not induce tumors when inoculated into newborn BALB/c and NIH strains of Swiss mice. A more sensitive test for MSV is its focus-inducing ability on mouse embryo cells

TABLE I. Influence of Neonatal Thymectomy on Tumor Induction by MSV.

Strain	Age virus injected (days)	No. of rats with tumors / No. of rats inoculated: Takes	Prog. growth	Avg latent period and range (days)
BN (thy)	2–3	6/10	4/10	22 (18–34)
" (intact)	"	0/5	0/5	—
Lewis (thy)	2–3	3/11	2/11	31.5 (26, 37)
" (intact)	"	0/5	0/5	—
BN (thy)	1	6/14	6/14	21 (16–32)
" (intact)	"	1/7	1/7	26
" (thy)	7–9	0/6	0/6	—
Lewis (thy)	1	10/21	10/21	23 (18–31)
" (intact)	"	0/22	0/22	—
(BN × Lewis) F_1 (thy)	1	1/6	1/6	33
" (intact)	"	0/4	0/4	—
" (thy)	7	0/4	0/4	—
" (intact)	"	0/4	0/4	—

(MEC)(7). This test also gave negative results. MSB-1 cells also did not react in the CF test with broadly reactive sera of rats immunized with Moloney lymphoma.

Recently Huebner *et al* have described a virus-free hamster tumor (HT-1) induced originally by MSV(9). Infectious MSV was recovered from the mixed culture of HT-1 cells and mouse embryo cells when superinfected with leukemogenic viruses. These authors concluded that the defective genome of MSV persists in HT-1 cells, transfers to the mouse cells and is propagated along with the superinfecting leukemogenic virus which also acts as a helper virus for the replication of MSV in mouse cells. Similar tests designed to rescue defective MSV genome in MSB-1 cells (Table II) showed that infectious MSV can be recovered from mixed culture of MSB-1 and mouse embryo cells, but also directly from MSB-1 cells by superinfection of leukemogenic viruses. The availability of this system now makes it possible to assay leukemogenic viruses quantitatively by their rescuing activity (RA). Preliminary results in Table III showed that Rauscher virus released from leukemic rat kidney cells has a titer of rescuing activity of 5×10^4 RA/ml. For a period of 6 months, only 2 out of 10 OM

TABLE II. Recovery of MSV Genome from Virus-Free MSB-1 Tumor Cells by Superinfection of Murine Leukemogenic Viruses.

Culture	Superinfecting virus	FFU/plate (0.2 ml in fluid harvested at various transfers*) 0	1	2	3
MSB-1	None	0	0	0	0
”	MLV	0	0	0	4,3,3
”	RV	0	0	4,5,5	72,116, 103
MSB-1+MEC	None	0	0	0	0
”	MLV	0	9,11, 13	Confluent	N.T.†
”	RV	1,3, 1	69,72, 63	Confluent	N.T.
MEC	None	0	0	0	0
”	MLV	0	0	0	0
”	RV	0	0	0	0
BNEC‡	None	0	0	0	0
”	MLV	0	0	0	0
”	RV	0	0	0	0

* Cultures transferred every 3 days.
† N.T. = Not tested.
‡ BNEC = BN rat embryo cells.

TABLE III. Assay of Rauscher Virus for Its Rescuing Activity of MSV Genome in Mixed Culture of MSB-1 Cells and Mouse Embryo Cells.

Dilution of virus	Avg FFU/plate* (0.2 ml in fluid harvested at various transfers†) 0	1	2	3	4	5
Undiluted	2	>100	>100	N.T.‡	N.T.	N.T.
10^{-1}	0.66	>100	>100	N.T.	N.T.	N.T.
10^{-2}	0	71	>100	N.T.	N.T.	N.T.
10^{-3}	0	6.3	64	N.T.	N.T.	N.T.
10^{-4}	0	0	0	0	0.33	51
10^{-5}	0	0	0	0	0	0
10^{-6}	0	0	0	0	0	0

* Average value of 3 plates.
† Cultures transferred every 3 days.
‡ N.T. = Not tested.

rats which have received 0.1 ml of the same undiluted preparation as newborn developed thymic leukemia. A correlation study of infectivity, RA titer, CF titer and particle count is now underway.

Abundant evidence has been accumulated indicating that neoplasms induced by several oncogenic viruses possess specific transplantation antigens common for different tumors induced by the same virus(10). Transplantation resistance has been reported in mouse leukemias induced by Gross, Friend, MLV and Rauscher viruses(11,12,13). Since all virus-induced lymphomas release viruses continuously, it is not known whether the immunogen is cellular or viral. Virus-free MSB-1 cells were therefore used to test whether they possess tumor specific antigen. Adult BN rats were given various kinds of tumor suspension as follows: 2×10^6 trysinized cells suspended in tris buffer were injected subcutaneously; 2nd and 3rd injections were given at an interval of every 2 weeks. Two weeks after last injection, all rats were challenged subcutaneously with MSB-1 cells. Adult BN rats infected with MSV and MLV were challenged 4 weeks later. Results of these experiments are shown in Table IV. It is evident that MSV, MLV, MSV tumor induced in Lewis rat and leukemia induced in OM rat by MLV protected rats against challenge of MSB-1 cells whereas polyoma tumor and Schmidt-Ruppin-Rous tumors did not. It should be noted here that MSL-2 tumor is also a virus-free tumor originally induced by MSV in Lewis rats. It is not surprising that lymphoma in OM rats which

TABLE IV. Growth of MSB-1 Tumor Cells in Adult BN Rats.

		No. of BN rats with tumors / No. of BN rats challenged (Avg time of death and range in days)		
		MSB-1 cells challenge dose		
Exp No.	Treatment	5×10^5	5×10^4	5×10^3
1	Control	5/5 (24 : 14–33)	4/5 (31 : 19–53)	3/5 (55 : 44–57)
	MSV	4/5 (48 : 41–60)	1/5 (66)	0/5
	MLV	5/5 (34 : 26–44)	2/5 (45 : 39–52)	0/5
2	Control	5/5 (17 : 16–20)	5/5 (29 : 20–27)	3/5 (53 : 34–62)
	SR tumor*	5/5 (15 : 14–18)	5/5 (19 : 16–20)	2/5 (41 : 35–47)
	Py tumor†	5/5 (22 : 14–27)	5/5 (24 : 20–31)	4/5 (49 : 27–74)
	ML tumor‡	4/5 (30 : 27–33)	2/5 (37 : 35–39)	0/5
	MSTC5§	3/5 (44 : 28–60)	1/5 (67)	0/5
	MSL-2‖	4/5 (46 : 37–67)	1/5 (69)	0/5

* Schmidt-Ruppin Rous sarcoma in Amsterdam rat.
† Polyoma tumor in Lewis rat.
‡ Moloney lymphoma in OM rat.
§ BN embryo cells infected with MSV in culture(2).
‖ MSV tumor in Lewis rat.

releases MLV and MLV alone can also induce resistance in BN rats against the challenge of MSB-1 cells, since MSV was originally isolated from MLV stock and the focus-inducing ability of MSV requires the help of MLV (7). However, it is not certain whether MSV is a mutant of MLV.

Discussion. It is evident that transplantation resistance antigen(s) can now be demonstrated in virus-free MSB-1 tumor cells. Though MSB-1 cells do not release infectious MSV or MLV, the genome of MSV can be easily rescued by superinfection with leukemogenic viruses. In contrast to hamster tumor cells, we are able to rescue the MSV genome directly from MSB-1 cells; this may be due to the inability of the leukemia virus to propagate in hamster cells.

Results reported here also indicate that titration of rescuing activity is a sensitive and quantitative method for determining biological activity of leukemogenic viruses, especially those which may have low infectivity in animals because of attenuation by growing in tissue culture cells.

Summary. Murine Sarcoma Virus (Moloney) rarely induces tumors in intact newborn rats. When rats were thymectomized at birth and infected within 2-3 days, 30 to 50% of infected thymectomized rats developed tumors. One of the transplantable tumors established in tissue culture is found to be free of infectious virus. It possesses tumor specific antigen as demonstrated by transplantation resistance tests. Furthermore the defective sarcoma viral genome can be rescued either by superinfection of a mixed culture of tumor cells and mouse embryo cells with leukemogenic viruses or by direct superinfection of tumor cells. It is now possible to assay the rescuing-activity quantitatively with given preparation of leukemogenic virus.

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