Table 1. Tumor production in turkeys by implanted Rous sarcoma (Rs) cells.

Age (day)		Cells* implanted			Tumor response	
Cell recipient	Cell donor†	Kind	Type of implant	Virus titer‡	Tu/total§	Diameter (mm)
7	27–29	Rs	Homologous	1.9–3.5	32/32	15–30
7	27–29	Rs	Homologous	2.4–3.5	26/26	15–30
7¶	27–29¶	Rs¶	Homologous¶	2.4–3.5¶	16/26¶	3–10¶
7	27–67	Rs	Homologous	<0.3	2/35	3
7	27	Normal‖	Homologous	<0.3	0/15	0
35	34–44	Rs	Homologous	<0.3	8/32	3–10
34–44	34–44	Rs	Isologous	<0.3	8/26	5–10
35			Cell-free virus	2.7	12/12	>10

* 3 × 10^5 to 6 × 10^6 cells per bird. † Tumors produced by inoculation with virus at the age of 7 days. ‡ Log PFU per 0.2 milliliter. § Tu/total = number birds developing tumors / total number birds inoculated. ¶ The same conditions, but antivital antiserum (turkey immune serum diluted 1:10) was also administered. See text. ‖ Control turkey normal wing web cells.

units (PFU) of virus. A few tumors were initiated by inoculation of 2 PFU or less of virus. At various times after infection, birds with well-developed wing tumors were killed, and their tumors were aseptically dissected. The tumor tissue was then processed as previously described (*5*). The number of cells in the resulting cell suspension was determined by staining with trypan blue and counting with a hemocytometer. Only viable cells were counted. In addition, the cells were grown in tissue culture to confirm their viability. A measured sample of the cell suspension was sonically disrupted, and the virus content was determined by assay in embryonated hen eggs (*6*). Another sample of intact cells of the same suspension was used for inoculation into the left wing web of turkeys. Groups of 10 to 15 turkeys were each inoculated with 0.2 ml of a cell suspension. In cases of isologous cell transfer, the bird was placed under light ether anesthesia, a piece of tumor was biopsied and processed by the same procedure, and the resulting cell suspension was reinoculated into the opposite wing web of the same donor bird from which the tumor cells were obtained. After inoculation the birds were examined daily for 35 days, and their tumor sizes were recorded.

Table 1 shows that only those tumor cells that carried appreciable amounts of infectious virus produced tumors in all of the birds inoculated. These tumors grew rapidly and reached a large size; they did not regress within the 35-day period of observation. Exposure of such cells to antiviral antibody prior to implantation into birds resulted in a 38-percent reduction of tumor incidence. Further, the tumors that did appear were small and grew at a slower rate than tumors induced in the absence of antiviral antiserum. The reduction of tumor incidence was even more pronounced if cells that contained less than log 0.3 pock-forming units of virus were implanted. Such cells were obtained from birds with tumors produced with both high and low infecting doses of virus, and all such birds possessed high levels of circulating antiviral antibody (that is, they had a serum neutralization index of 10^3 to 10^5 when diluted 1:10). The few tumors that appeared were very small and did not exhibit progressive growth. Some regressed within 5 weeks. Similar results were obtained with isologous transfers of cells. No marked increase in tumor incidence or growth rate was observed and regressions also occurred. The data thus show that Rous sarcoma cells are not readily transplantable and the tumors produced by cell implantation appear to be largely virus-induced. Indeed, even those few tumors that resulted from implantation of cells carrying less than log 0.3 pock-forming units of virus appeared to be virus-induced, since their incidence resembled the incidence encountered when low doses of cell-free virus were used to produce tumors (*7*). The possibility cannot be excluded that a small initial proliferation of the implanted cells did occur. However, it is quite clear that, even if this was the case, it rarely resulted, if at all, in grossly visible tumors. These observations implicate virus and infection of host cells as the major factor in causing malignant, progressive growth of Rous sarcoma transplants in turkeys.

It seems possible, especially in view of recent findings on the pathogenesis of virus-induced Rous sarcoma (*8*), that infectious virus may be essential for maintaining unlimited growth and secondary spread of established tumors as well. If so, immunity to the virus could conceivably limit the growth and systemic dissemination of these neoplasms.

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Generalized Shwartzman Reaction in the Pregnant Golden Hamster

Abstract. *A single intraperitoneal injection of colchicine regularly elicits the generalized Shwartzman phenomenon in the pregnant golden hamster. Nonpregnant hamsters are refractory both to colchicine and endotoxin. The superiority of colchicine, as opposed to foreign endotoxin, in the pregnant subject, may be attributed to the action of native endotoxin which is allowed parenteral access as a result of colchicine-induced injury to the intestinal mucosa.*

In the course of experiments designed to study the hamster karyotype, it was observed that an intraperitoneal injection of colchicine (150 to 300 mg/kg of body weight) induced a severe reaction in pregnant golden hamsters, although it had relatively little effect in nonpregnant individuals. The pregnant animals became lethargic, exhibited extensor convulsions, and began to abort, with bleeding from the vagina. During the maximum period of observation of 24 hours, the general condition inexorably deteriorated, although death did not ensue. Systematic examination of all animals was performed under Nembutal anesthesia. The renal surface was mottled and finely irregular, with occasional petechial hemorrhages. Accentuation of the lobular pattern of the liver,

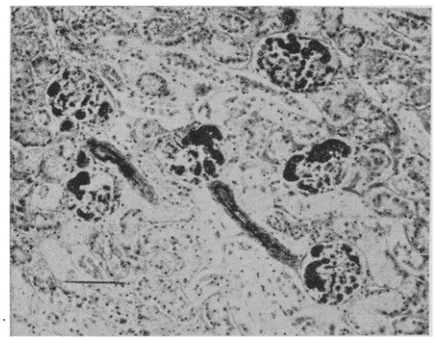

Fig. 1. Renal cortex showing marked fibrin deposition in the glomerular capillaries and early tubular necrosis (PTAH stain; scale, 100 μ).

adrenal hemorrhage, and moderate pulmonary congestion with punctate hemorrhages were less frequently encountered. The uterus invariably presented a dark appearance, owing to contained hemolyzed blood originating from placental hemorrhage. Early maceration of the fetuses was a constant feature. Portions of all tissues were fixed in 10-percent formalin, embedded in paraffin, sectioned at 7μ, and stained with hematoxylin and eosin, phosphotungstic acid hematoxylin (PTAH), and other stains. The most conspicuous feature on histological examination was the presence of a fibrillar substance blocking the renal glomerular capillaries (Fig. 1), which was identified as fibrin, on the basis of the characteristic deep-blue staining by PTAH. This finding is pathognomonic of the generalized Shwartzman reaction (*1*). Fibrin thrombi were less frequently present in the lungs, brain, spleen, liver, and adrenals. The incidence of renal glomerular fibrin thrombosis is shown in Table 1. Although the overall architecture of the intestinal mucosa was unaffected, considerable damage to the epithelium was evident in the form of disruption of cells arrested in mitosis.

Table 1. Incidence of fibrin thrombi in renal glomerular capillaries. Ratios are number of animals affected to number of animals tested.

Provocative agent	Fibrin thrombi
Pregnant hamsters	
Colchicine*	6/7
Endotoxin†	1/6
Nonpregnant hamsters (♀ and ♂)	
Colchicine‡	0/8
Endotoxin§	0/30

* Colchicine, U.S.P. (Fisher), 150 to 300 mg/kg, intraperitoneally. † Endotoxin (Bacto lipopolysaccharide, *E. coli* 055:B5, Difco), 0.1 to 0.2 mg, intraperitoneally. ‡ 300 mg/kg, intraperitoneally (twice.) § Several schedules of administration, from 0.1 mg, intraperitoneally (twice) to 0.6 mg, intraperitoneally (once).

The generalized Shwartzman phenomenon is classically produced in the nonpregnant rabbit by two suitably spaced injections of bacterial endotoxin (*1*), and it has also been described in man (*2*) and in the pregnant rat (*3*). Attempts in this laboratory to reproduce the phenomenon in the nonpregnant hamster with two spaced doses of either endotoxin or colchicine were entirely unsuccessful. Endotoxin (0.2 mg, intraperitoneally) was only feebly effective in the pregnant hamster (Table 1) although the particular batch of endotoxin used possessed good Shwartzman-provoking activity in the pregnant rabbit. Administration of colchicine (300 mg/kg of body weight, intraperitoneally) to male hamsters carrying a choriocarcinoma of human origin in the wall of the cheek pouch resulted in the development of profound lethargy which was prevented by excision of the tumor immediately prior to the injection of colchicine. However, fibrin thrombi were not found. Tumor-bearing hamsters given endotoxin (0.2 mg, intraperitoneally) also became more shocked than the appropriate controls, but again fibrin thrombi failed to appear. Neither colchicine nor endotoxin, in two spaced doses, elicited the generalized Shwartzman phenomenon in the pregnant mouse, although several inbred strains and their hybrids were tested. The simultaneous administration of complement in the form of pooled guinea-pig serum failed to influence this negative result.

While it is generally conceded that intravascular coagulation, and in particular fibrin thrombosis in the renal glomerular capillaries, may be the determining event in the pathogenesis of the generalized Shwartzman reaction, there is less agreement concerning the nature of the state of preparation. Comparison of the susceptible pregnant hamster with the apparently unresponsive nonpregnant individual may yield information on this latter point.

The unique method of provocation may result from the loss of integrity of the intestinal mucosa produced as a consequence of the colchicine-induced mitotic arrest, thus permitting endotoxin derived from the native gut flora to enter the body. Adult members of a number of species possess demonstrable immunity to the Gram-negative bacilli composing their gut flora (*4*) and a suitable state of immunologic sensitivity directed towards the provocative agent may be a prerequisite for the successful precipitation of the generalized Shwartzman phenomenon in the prepared animal (*5*). An alternative mechanism may be connected with the liberation of the etiologic agent by the damaged placenta, although the relatively weak Shwartzman-provoking activity of endotoxin would tend to discount this possibility, as endotoxin, which is known to possess a powerful abortifacient action (*6*), gave rise to considerable placental injury in the present series (*7*).

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Deoxyribonuclease Sensitivity of Ribonucleic Acid Synthesizing System from Tobacco Leaves

Abstract. *A fraction from tobacco leaf cells, containing nuclei and capable of synthesizing ribonucleic acid in the presence of the four ribonucleoside triphosphates, was inactive after incubation with deoxyribonuclease, suggesting that deoxyribonucleic acid plays an essential role in the reaction. Almost complete inhibition was obtained even with concentrations of deoxyribonuclease which removed less than half the original acid-insoluble deoxyribonucleic acid.*

In a recent report, Bandurski and Maheshwari (*1*) demonstrated the ability of a nuclear fraction from tobacco leaves to incorporate nucleoside triphosphates into ribonucleic acid (RNA). RNA-synthesizing systems have been obtained from pea embryos, animal cells, and bacteria (*2*), and they are dependent upon deoxyribonucleic acid (DNA) for activity.