inoculated and non-inoculated mice were marked and kept together. Presence of tumors was investigated by macroscopical examination and palpation. Mice with tumors were autopsized, as were all mice 12 months after inoculation (Table).

Results and discussion. Tumors were observed after 8 months in both males and females. Table 1 summarizes the results. It is seen that tumor bearing animals were observed among the Visna virus infected Swiss albinos, as well as among the corresponding uninoculated or saline inoculated control animals. About 77% of the Visna virus infected female animals had tumors, while tumors were seen in 20% (p 0.001) of the female controls of Swiss albinos.

Incidence of tumors in Visna virus inoculated Swiss albino mice

Exp	Visna inoculated		Controls	
	Males	Females	Males	Females
1	0/13	8/14	0/12	1/12
2	3/14	22/25	1/7	4/13
1 + 2	3/27	30/39	1/19	5/25

Tumors of the mammary carcinoma type were observed in 39 mice and in 3 cases leukemias with enlarged spleens or livers were seen. Attempts to isolate Visna virus from tissues or blood of tumor-bearing animals by inoculation of plexus choroideus cells of sheep all failed.

About 10% of virus inoculated male Swiss albinos developed tumors, while tumors were found in 5% of male controls. The tumors seen in the males were cutaneous papillomas and leukemia.

In contrast, no increased incidence of tumors was observed among groups of 22 Visna virus-inoculated inbred BALB/c, CBA and DBA mice. Two of the virus-inoculated and two uninoculated mice died during the observation period and in one of the virus-inoculated animals, liver cysts were demonstrable.

Visna virus itself might not be directly responsible for the oncogenic effect observed. Increased incidence of mammary tumors was noted for the Swiss albinos but not for the mice of inbred strains with low incidence of spontaneously developed tumors. The above findings are consistent with preliminary experimental observations that inoculation of Visna virus in mice causes alterations in the immune system, i.e. functional changes in humoral and cell-mediated immunity. This will be reported in a following publication.

Differences in Susceptibility of Tissues to Revascularization Studied in Ectopic Implants

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Summary. The survival rate of implanted marrow is reduced when the tissue is transferred from one site to another within the first 24 hours but not more than 48 hours after the initial implantation. Splenic implants can be transferred at any time after implantation without affecting the survival rate. The observation suggests a difference in the sensitivity of the two tissues to the avascular period that they experience prior to initiation of angiogenesis.

Angiogenesis is a major limiting factor in growth, repair and regeneration of tissues. When the rate of tissue growth outpaces that of new vessel formation, circulatory insufficiency develops and results in necrosis or limitation of growth[2]. The autoimplantation of tissue pieces has served as a useful model to study the angiogenic potential of various normal tissues[2]. When tissues are so implanted, their normal circulation is severed and a successful take depends on new vessel growth with revascularization of the autoimplants. Implants of certain tissues, such as liver[2] and kidney[3] are not capable of eliciting revascularization; necrosis, typically of the coagulative type, supervenes. Implants of skin and bone marrow, on the other hand, show a great potential for angiogenesis[2]. These tissues are revascularized within 24 h after implantation ensuring a successful take in almost every experiment.

We have previously reported that spleen implants, although incapable of eliciting rapid angiogenesis, survive in ectopic sites[4], suggesting that the vulnerability of various tissues to the initial avascular period may also differ. In the present study an attempt was made to delay the revascularization of bone marrow and spleen implants to determine the vulnerability of these tissues to an initial avascular period. This was done by reimplanting the tissues in a new site at various intervals after initial implantation.

Materials and methods. Male Wistar rats (200–300 g) were used and anesthesia was given by i.p. injection of pentobarbital. The methods for ectopic implantation of marrow and spleen have previously been described[2,4]. Briefly, the knee joint was exposed and an opening made in the shaft of the femur, through its articular surface, using a low speed dental drill. A polyethylene tube (No 160) was then inserted into the shaft, the free end clamped and the tube then withdrawn. The marrow tissue, now filling the tube was expelled and implanted into a pocket made in the subcutaneous tissue of the abdomen through a midline incision. Fragments of splenic tissue 5 to 8 mm in diameter, were similarly implanted after the animals were splenectomized.

At various intervals the implants were removed and reimplanted, subcutaneously, on the opposite side of the midline abdominal incision. 5 or 6 weeks after the initial implantation the implants were studied histologically, and sequential studies of implants were made when indicated. The criterion of a successful take was the formation of normal-appearing splenic tissue or bone marrow nodule.

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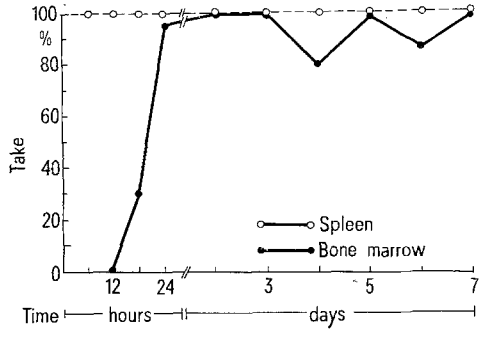
Results. The sequence of events associated with implantation of marrow and spleen have previously been described. Normally, marrow implants elicit an intense vascular reaction in the surrounding tissue. Vascular buds invade the implant so that within 24 h a defined border cannot be identified between the implant and the surrounding tissue. By day 3 to 4, the implant consists of proliferating fibroblasts interspersed with large numbers of capillaries giving the overall morphological appearance of granulation tissue. Through a well-defined sequence of histogenetic events, this granulation tissue develops into a hemopoietic nodule surrounded by a shell of bone. In contrast, the spleen implant elicits a slow vascular reaction in the supporting tissue bed. By 48 h the implant is not yet invaded by vascular buds and a sharply-defined border separates it from the implant. At this stage the implant can easliy be detached from the supporting bed without inducing hemorrhage from the surrounding tissue. The first vascular buds can be seen in the implanted splenic tissue 3–4 days after implantation (removal of the implant now causes some bleeding from the surrounding tissue). These vascular buds penetrate the implant in a centripetal direction, apparently triggering the proliferation of structural elements of spleen and reconstituting the new structure of spleen. The end product is a nodule of spleen histologically indistinguishable from the original tissue.

The Figure shows the survival of implants, removed at various intervals after initial implantation and reimplanted in a different subcutaneous site. It can be seen that reimplantation does not alter the survival of splenic implants irrespective of the interval between initial and subsequent implantation. For bone marrow, however, reimplantation during the first 24 h after the initial implantation significantly reduces the survival rate of the implants. This reduction is most pronounced if reimplantation is done during the first 12 h after initial implantation when the survival is reduced to zero.



Survival of marrow and spleen autoimplants transferred to new sites at various times following the initial implantation.
The vertical axis shows the survival of tissue as defined by the formation of normal appearing marrow nodules or spleen tissue 5–6 weeks after the initial implantation. The horizontal axis shows the time elapsed between the initial implantation and the subsequent reimplantation of tissue to a new site.

Discussion. The grafting of a large organ (liver, kidney, heart) involves anastamosis of its blood vessels to the general circulation[2]. This is possible when the organ's nutrient vessels are large enough to make the operation technically feasible. For smaller organs whose nutrient vessels are too dispersed (skin, endocrine glands) successful grafting must rely on the potential of the tissue to elicit angiogenesis. Proliferation of vascular endothelium is initiated by 'angiogenic factors' presumably derived from the implanted tissue. Most of the information concerning angiogenic factors has been derived from study of tumor cell systems, hence the term 'tumor angiogenic factor' (TAF)[5]. TAF is soluble and partial characterization has indicated that RNA and protein as its major components[6]. It is mitogenic for endothelial cells[6] and can be inhibited by neonatal cartilage[7].

It has previously been shown that the angiogenic potential of different tissues differs and this variability determines the potential of a given tissue to survive when implanted in ectopic sites. The experiments described in this study indicate that the potential for elaboration of angiogenic factor is not the only factor determining the survival or implanted tissue. For a variable time after implantation, until a new circulation is established, the transplanted tissue is deprived of blood flow, and the implanted tissue must be able to survive this 'avascular phase'. When the implanted tissue is reimplanted the mitogenic effect of angiogenic factor on vascular proliferation begins anew so that the avascular phase is prolonged. The survival of reimplanted tissue, therefore, depends on its withstanding this extended avascular phase, when reimplantation is done before the process of regeneration has begun. 24 h after initial implantation, when the regenerative process has been initiated, the bone marrow tissue is no longer so vulnerable, and transfer of the tissue at this stage does not significantly reduce the survival rate. Splenic tissue, on the other hand, is not vulnerable to prolongation of the avascular phase. Reimplantation at anytime during the process of regeneration does not influence the survival rates.

The reimplantation of ectopic tissue implants provides a useful experimental means to evaluate the vulnerability transplanted tissues to periods of ischemia prior to establishment by angiogenesis of an effective circulation.

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Induction of Fibrosarcoma by Administration of DMBA into Mandibular Sockets of Rats

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3 November 1975.

Summary. DMBA in a slow releasing vehicle was implanted into mandibular sockets after extraction of rat molars. Fibrosarcomas developed within 9 months.

Ample evidence has accumulated on the subject of experimental carcinogenesis in oral tissues of laboratory animals. DMBA (9,10-dimethyl-1,2-benzanthracene) has been commonly used for this purpose. It was introduced in the pure form, in cholesterol pellets and in gelatin capsules, or it was dissolved in acetone or olive oil and injected or instilled into the salivary glands. DMBA-induced neoplasms, developed in connective tissue, squamous and glandular epithelium of rats, mice, guinea-pigs and hamsters, comprizing sarcomas, epidemoid and