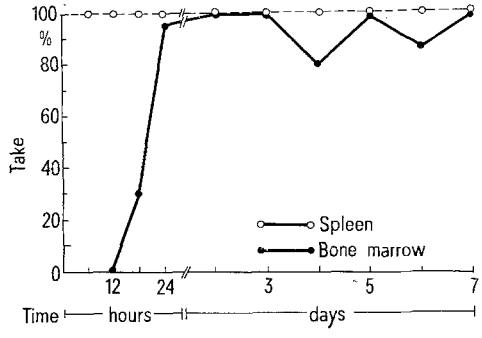
Results. The sequence of events associated with implantation of marrow and spleen have previously been described. Normally, marrow implants elicit an intense vascular reaction in the surrounding tissue. Vascular buds invade the implant so that within 24 h a defined border cannot be identified between 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

adenocarcinomas[1-12]. The purpose of the present study was to investigate the effect of DMBA, in a slow releasing vehicle, on the tissues involved in socket healing, following extraction of mandibular molars in rats.

30 albino rats of either sex of the Hebrew University (Sabra) strain, weighing 100 g each, were used. The animals were anesthetized and the mandibular first right molars were extracted. Pellets of 0.2 ml Vitepsol H-15 (Dynamit Nobel AG, 5000 Köln-Mülheim 1, W. Germany) containing 10% DMBA were introduced into the socket of 10 rats, sutures were placed in order to prevent the removal of the carcinogen. Another 2 emulsified pellets (at 35 °C) were injected into the operation site, the first after 10 days and the second 20 days following extraction. 20 rats served as controls, they were subjected to a similar treatment schedule. In 10 rats, the sockets were rinsed with saline which was also injected twice into the healing area. Another 10 animals received pellets of 0.2 ml Vitepsol H-15 into the sockets; injections of the emulsified vehicle completed the treatment. The animals were kept under surveillance and roentgenographed periodically. They were killed after 9 months, the jaws were fixed in formalin. Paraffin embedded sections were cut at 6 µm and stained with hematoxylin-eosin and Masson's Trichrome method for connective tissue.

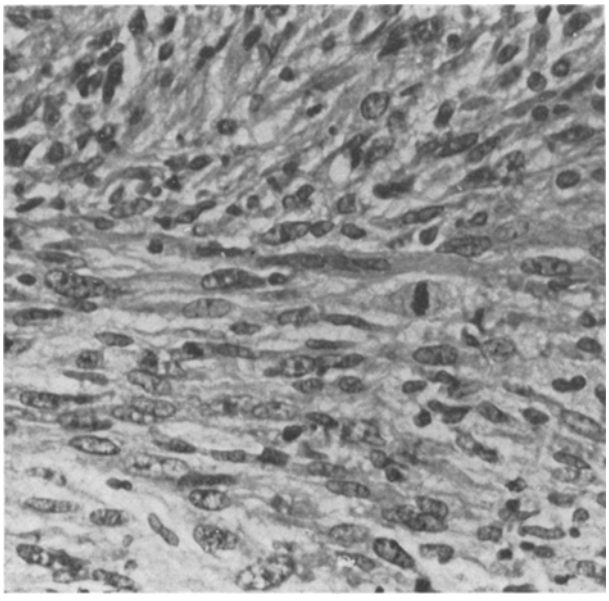

Fig. 1. Fibrosarcoma, note anaplasia of cells, variation in size and shape of nuclei. Hematoxylin and Eosin. × 320.

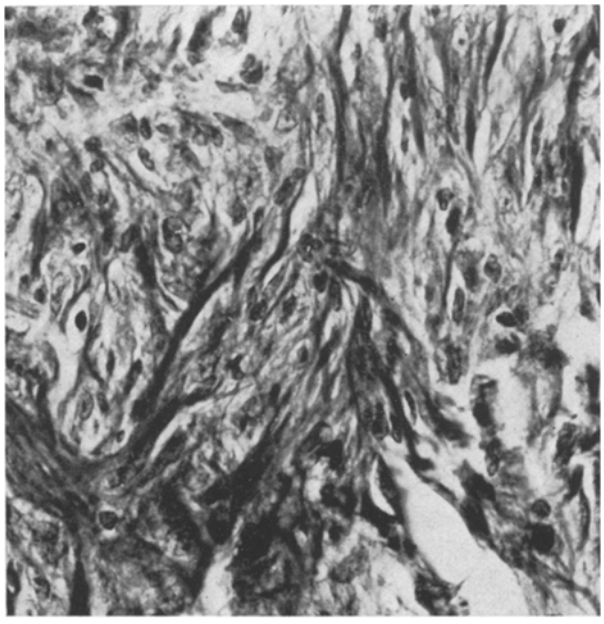

Fig. 2. The neoplasm is characterized by the formation of interlacing bundles of collagen fibres. Masson's Trichrome, × 320.

Six of the 10 DMBA-treated rats developed tumor masses, the diameters of these swellings ranged from 4 to 6 cm. The neoplasms derived from the sockets and invaded the adjacent tissues. Histological evaluation revealed that in all cases the tumors were fibrosarcomas (Figure 1). This was also supported by positively stained fibriles with Masson's Trichrome method for connective tissue (Figure 2). 1 of the 6 rats developed a papilloma in addition to the primary tumor, i.e., fibrosarcoma. 2 rats died spontaneously 4 and 6 months after the operation and another 2 did not develop neoplasms. Examination of the 20 control rats revealed that none of these animals developed any neoplastic growth.

The method described herein was shown to be operative in induction of fibrosarcoma by the administration of DMBA into the socket of rat mandibular molars following extraction. The carcinogen was applied in Vitepsol H-15 pellets in order to induce a prolonged action. It was placed into the bone immediately after the injury and operated during the remodelling process on the mesenchymal tissues involved in healing. The importance of the continuous action of a carcinogen was indicated in the past[13]. Carcinoma developed following introduction of the material into an artificial cecal epithelial-lined pouch in lips of rats. The present method, however, was designed to develop osteosarcoma by implantation of DMBA in a slow releasing vehicle into the bone, following the extraction of molars. As with other experiments referred to previously, no osteosarcomas developed. The constant production of sarcomas with the experimental procedure described herein, makes it highly recommendable. Yet, further evaluation of this method aiming at the induction of osteosarcoma, is required. Implantation of methylcholanthrene in artificial defects drilled in the incisor root area of mice, induced osteosarcoma[14]. The diversity of previous results as compared to the present study may be due to the difference of animal species and types of carcinogens used. It is of interest that the duration of the experiment i.e., 9 months, was identical in both studies. Perusal of the literature (including a search of the W.H.O. Medline Center) did not reveal reports on an experimental model similar to that described herein.

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