joint did not appear in the other tissue site, while, on the other hand, PPLO in one tumor were transmitted to another tumor. Furthermore, the varied time of appearance of separate arthritic lesions suggest non-contiguous dissemination from one joint to another. To the uncertain roles of local suppuration and of the anti-PPLO antibodies in transmission of PPLO in the above circumstances, one may add the role of tumor tissue *per se*—a remarkable milieu in which anigen-antibody and inflammatory responses, quite unlike those in other tissues, would not be unexpected.

Summary. Mycoplasma (PPLO) transmitted with the Murphy-Sturm lymphosarcoma at time of transplant, a) did not modify tumor growth, b) mildly increased the low incidence of spontaneous regression of the tumor and, c) increased the regression due to bacterial polysaccharide and to established PPLO polyarthritis. Following their intravenous injection, PPLO were readily cultured from tumor tissue. When injected prior to transplant of tumor cells, persistent polyarthritis developed but PPLO did not appear in the subsequent tumor. However, when 2 tumors, one infected and one free of PPLO, were transplanted to the same rat, PPLO appeared in the original PPLO-free tumor after moderate tumor size was attained. Agar plate cultures of PPLO indicated several colony sizes and an occasional diffuse surface spread.

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Comparison of Autologous, Homologous, and Heterologous Normal Skin Grafts in the Hamster Cheek Pouch.* (26440)

STANLEY N. COHEN† (Introduced by J. R. Brobeck)
Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia

During recent years, the cheek pouch of the Syrian hamster has become an increasingly popular site for transplantation of fetal (1,2), malignant(3,4,5,6), and normal adult (7) tissues. Toolan(8) has observed increased growth potentialities of normal heterologous tissues implanted into the cheek pouch of the cortisonized hamster. Lemon, *et al.*(3) have reported the persistence of some normal human tissues in the cheek pouch of untreated hamsters but have failed to observe definite evidence of growth. It appeared desirable to evaluate carefully the cheek pouch as a site for grafted tissues and to compare the behavior of normal autologous, homologous, and heterologous grafts in both cortisonized and unconditioned hosts.

Methods. Male young adult Syrian hamsters, from a non-inbred colony, weighing between 70 and 100 g were used in all of these experiments. The animals were implanted with 1 mm square portions of normal skin according to the method of cheek pouch implantation described by Lutz, *et al.*(9). The entire thickness of abdominal skin was taken for the hamster homografts and autografts. For the heterografts, a 1 mm thickness of

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† Present address: Mount Sinai Hospital, New York.

TABLE I. Survival of Skin Grafts in Hamster Cheek Pouch.

Type of graft	Sacrificed 3 wk after implantation				Sacrificed 6 wk after implantation			
	Cortisonized		Unconditioned		Cortisonized		Unconditioned	
	No. of implants	No. surviving	No. of implants	No. surviving	No. of implants	No. surviving	No. of implants	No. surviving
Heterologous	13	10	14	7	12	9	13	6
Homologous	11	7	13	12	12	7	11	9
Autologous	12	6	11	9	14	8	12	12

adult rabbit skin freshly obtained from the back of a black-haired strain and removed from an area in the inactive phase of the hair growth cycle was employed. The skin was shaved, then wiped with dry sterile gauze before removing it. The grafts were not treated in any other way.

Bilateral implantations were employed, and all 3 possible combinations were studied —*i.e.*, auto-homo, auto-hetero, and homo-hetero. Half the recipient animals received 3 mg of cortisone acetate (Merck) subcutaneously in the nape of the neck at time of implantation and 2 mg twice weekly for the remainder of experiment. The remaining animals were untreated.

Animals were sacrificed after 3 and 6 weeks and degree of growth of the graft in the cheek pouch was determined by examination of histological specimens. The criteria employed in determining successful or unsuccessful survival were: mitotic activity, appendage formation (hair follicles, sebaceous glands), pigment formation, and migratory activity of the epidermis(10). Infection widespread enough to cause significant necrosis of the graft and/or host tissue occurred in approximately 10% of all implantations. In these instances, the section was discarded from the series. One of the 36 animals used died during the experiment and was not included in the series.

In addition to being rated microscopically as "surviving" or "rejected", sections were subjectively evaluated on a "blind" basis—using an arbitrary 0 to 4 scale—and employing degree of mitotic activity, appendage and pigment formation, and migratory activity as criteria. This procedure produced evaluations of surprising consistency over repeated trials.

Results. For 6-10 days after implantation, all grafts appeared grossly as yellowish-white nodules 3-8 mm in diameter surrounded by a variable area of increased vascularity. Surviving grafts retained this general appearance. Regressing transplants decreased in size after 6-10 days and about one-third was completely absorbed by 16-20 days. In the remaining two-thirds of regressing grafts, nodules 1-2 mm in diameter marking the site of a granulomatous reaction persisted for 2-4 weeks longer.

Survival of the 3 types of grafts is shown in Table I. Heterografts were surviving at 3 and 6 weeks in both cortisonized and untreated hamsters. The hair cycle, which was in the inactive phase at time of implantation, converted to the active phase—and mitoses, hair follicle and gland development, and pigment formation were seen in surviving grafts in both conditioned and untreated animals (Fig. 1, *top*). Epithelial migratory activity was marked (complete encystment by 3 weeks) in all surviving implants, and most of the necrotic implants showed evidence of partial encystment before being rejected. All cysts were of the "external"(11) type, with the cuticular layer facing the center of the cyst and the basal layer facing outward. (Fig. 1, *bottom*). The appearance of the grafts was not significantly different at 3 and 6 weeks. Subjective evaluation as well as survival-regression ratios indicated that heterografts were growing more successfully in cortisonized animals. There was no significant difference in the behavior of cheek pouch autografts and homografts from the same non-inbred colony (Table I). In contrast to the behavior of heterografts, both autografts and homografts grew more successfully in *non-cortisonized* animals. Indeed, there were

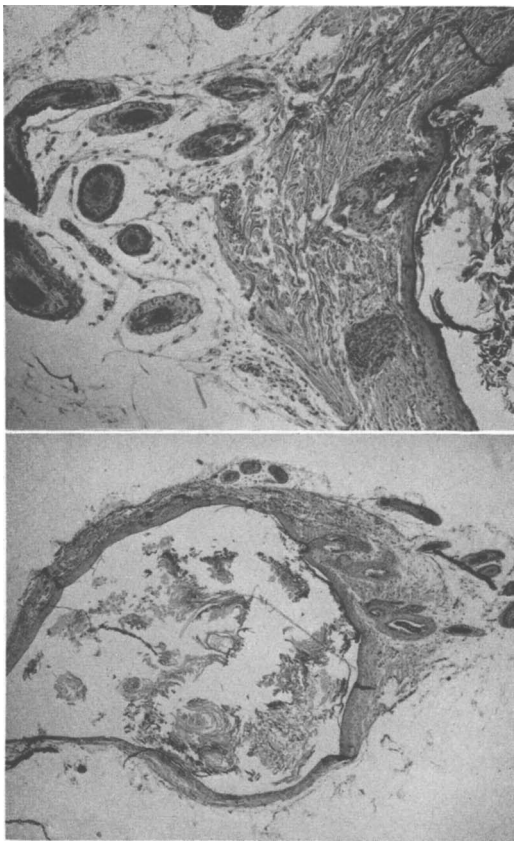

FIG. 1, *top.* Adult rabbit skin heterograft in cheek pouch of unconditioned hamster 42 days after implantation. ×72. Development of dermal appendages and pigment formation are evident.

FIG. 1, *bottom.* Adult rabbit skin heterograft in cheek pouch of unconditioned hamster 42 days after implantation. ×30. Complete "external" encystment has occurred via epithelial migration.

a surprisingly small number of "takes" in treated hamsters.

All rejected grafts were surrounded and infiltrated by large numbers of lymphocytes and plasma cells. In addition, smaller numbers of foreign body giant cells, monocytes, and polymorphonuclear elements were seen amidst the necrotic tissue. Lymphocytes and plasma cells in significant numbers surrounded and infiltrated 10 of the 13 surviving heterografts in untreated animals—but graft survival and growth occurred despite the presence of this infiltration. A lesser mononuclear cell accumulation was noted in surviving heterografts in cortisonized hamsters, and still fewer were seen in surviving autografts and homografts.

It should be noted that changing the kind of graft in one cheek pouch had no effect on the contralateral cheek pouch graft in the same animal. For example, heterografts grow equally well whether a homograft or an autograft was in the opposite cheek pouch.

In view of the surprising extent of heterograft survival in untreated hamsters, orthotopic heterografts of adult rabbit ear skin were placed on the flanks of 10 hamsters from the same non-inbred colony according to the method described by Billingham and Medawar(12). Four of the recipient animals received the same dose of cortisone given to cheek pouch implant recipients; the remainder were untreated.

In contrast with the results observed in the cheek pouch, these orthotopic heterografts were uniformly non-viable beyond the 7th day. Cortisone was completely ineffective in prolonging the survival of these grafts. Following initial vascularization, evident on the third or fourth day after implantation, hemorrhagic necrosis developed and the graft was rejected in the familiar fashion(12,13).

Discussion. A number of workers have reported growth of adult heterologous skin implants in the cheek pouch of the cortisonized hamster(5,3,7). Foley and Handler (3), working with tissue culture cell inocula, found that normal heterologous tissue injected in a sufficiently large inoculum will grow and proliferate for at least a short period of time in the hamster cheek pouch, even when no cortisone treatment of the recipient is employed. Billingham and Hildemann(13) have shown (and the experiments reported here have confirmed) that normal skin heterografts are promptly rejected from the body surface of the hamster in every instance —even with the use of cortisone, and have suggested that "the cheek pouch may be an immunologically privileged environment, where grafts either fail to elicit a maximal response or are to some extent exempt from its consequences." The experiments reported here (*i.e.*, survival and growth of adult skin heterografts in the hamster cheek pouch even without conditioning of the recipient) suggest that the cheek pouch *is* in some way immunologically privileged and that grafts placed

there are not subject to the same factors causing prompt rejection of orthotopic skin heterografts. These experiments suggest, however, that the sanctuary of the cheek pouch is not complete for, when heterografts are given further protection against rejection by use of cortisone, they grow more successfully than they do without the steroid.

The acceptance of intracolony orthotopic homografts by the hamster and the behavior of these like autografts has been reported by a number of investigators, and thus the survival of intracolony cheek-pouch homografts was not unexpected. It is interesting to note, however, that in contrast to heterografts, both auto and homografts demonstrated poorer survival in cortisonized animals than in untreated controls. The findings reported here are consistent with those reported by Vasiliev in rats(15), *i.e.*, that cortisone enhanced the growth of heterologous tumors but inhibited the growth of tumor homografts.

A word should be said about the marked lymphocytic response noted in *surviving* heterografts as well as in all necrotic implants. If one adheres to the current concept that lymphocytic elements are associated with the phenomenon of graft rejection, one is struck by the seeming impotence of these lymphocyte and plasma cell infiltrations amid the proliferating cheek pouch implants. This response was noted in those animals sacrificed at 3 weeks, and presumably had been present in the "6-week" group for at least 21 days without changing significantly in appearance or causing graft necrosis. It appears that a base-line lymphocytic infiltration occurs in response to all grafts (even autografts) but an increased number of these cells accumulates specifically in response to heterografts. This additional accumulation is inhibited by cortisone.

Experiments are presently underway to determine the basis of the cheek pouch's apparently privileged state.

Summary. 1) Adult rabbit-skin heterografts were capable of surviving at least 6 weeks and proliferating in cheek pouches of both cortisonized and untreated Syrian hamsters. Orthotopic heterografts were uniformly non-viable beyond the seventh day. 2) No difference in survival was observed between autografts and homografts from the same non-inbred colony. 3) A marked lymphocytic reaction was evident in all rejected grafts and in surviving heterografts. This response was inhibited by cortisone and apparently did not prevent growth of these heterografts. 4) In general, cortisone inhibited growth of homografts and autografts and enhanced growth of heterografts. 5) The nature of the graft in one cheek pouch had no effect on the growth of the contralateral graft.

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