genesis, but they do rule out any effect above a doubling of the spontaneous mutation frequency. To assay for such low-level effects, it would be more appropriate to test LSD with microbial systems (*Neurospora, Aspergillus, Saccharomyces,* bacteria) where populations of 10^7 or more treated cells have been assayed by selective techniques for antibiotic resistance, nutritional mutants, or reverse mutations (*11*).

It is our belief that the most satisfactory screening method for a definitive analysis of the genetic effects of LSD or other chemicals should be a three-step procedure: (i) mutagenesis tests with microbial organisms; (ii) mutagenesis and breakage tests with *Drosophila;* and (iii) cytological tests for breakage in small mammals (rats, hamsters, and so forth) whose genetic lineage can be rigorously controlled. The universality of the basic components of metabolism and the universality of the genetic coding machinery (*23*) make it unlikely that a dangerous mutagen would be missed by the three-step procedure we recommend. This procedure is similar to that recommended by Kaufmann and Schuler for pharmaceutical products (*24*). Because of the possible or proven genetic and teratogenic hazards associated with certain pharmaceuticals, antibiotics, preservatives, additives, and other chemical agents (*25*) we believe that the screening procedure we described should be used before these substances are made available to the public.

Our experiments in *Drosophila* do not substantiate the conclusion that LSD has any pronounced effect on genes and chromosomes. We recognize that high doses of LSD (varying from 100 μg to several thousand micrograms) induce strong psychological reactions in humans (*26*). For this reason we do not wish our results at the genetic level to be used as a justification for nonmedical use of LSD as a mind-altering chemical. We strongly recommend that the social debate on the uses and abuses of LSD be based on what is actually known, from rigorously controlled experiments, rather than from conjecture, insufficient sample size, isolated case histories lacking rigorous controls, and subjective individual experience.

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8 April 1968; revised 18 June 1968 ■

Trophoblast Antigenicity Demonstrated by Altered Challenge Graft Survival

Abstract. *Adult C57BL/6J male mice received either a primary ectopic transplant or a primary and a challenge ectopic transplant of trophoblast tissue obtained from the ectoplacental cones of 7½-day-old C3H/HeJ × C3H/HeJ embryos. Gross and histologic examinations of these grafts at 5, 7, and 12 days of growth indicated that the challenge grafts were inhibited in growth; there were a smaller percentage of grossly successful grafts, smaller hemorrhagic reactions, fewer viable cells at all stages of growth, and better host containment of the colony. The evidence indicates that exposure to pure trophoblast alters an animal's subsequent reaction to grafts syngeneic with the original trophoblast and can best be explained as evidence of immunologic sensitization of the host.*

In considering the immunologic relationship between a mother and her fetus, one must determine whether the trophoblast can be recognized by the mother as antigenic. Previous work has suggested that the allogeneic histocompatibility antigen is not expressed on the trophoblast (*1*), although xenogeneic antigen may be present (*2*). The possibility that an antigen specific to trophoblast exists has been suggested for humans (*3*) but has not been confirmed in animal studies. We now report observations of the effects of ectopic trophoblast allografts on the growth of subsequent trophoblast transplants.

Female mice (C3H/HeJ from Bar Harbor, Maine) were killed 7½ days after mating with syngeneic males. The embryos were removed from the deciduomata, and the ectoplacental cones were separated from embryonic and decidual tissue. Each cone was transplanted by means of a 22-gauge spinal needle into the subcapsular area of a kidney of a C57BL/6J male.

This methodology was chosen for a number of reasons. Earlier observations indicated that embryonic parts can develop into trophoblast colonies derived from 2½-day-old fertilized ova. The presence of such embryonic elements suggested that immunologic activity could be stimulated by tissues other

than trophoblast (*4*). The careful dissection of maternal decidua and fetal membranes from the 7½-day-old transplanted ectoplacental cones, plus the failure to observe any cells other than trophoblast growing, minimized if not eliminated this possibility. In previous studies several eggs or cones were transplanted at each graft site (*1*). Because some transplants fail to grow as a result of technical difficulties (about 25 percent in our studies), the use of several eggs or cones makes difficult judgment of the quantity of antigen responsible for observed phenomena. We controlled the dosage of antigen by transplanting one cone at a time.

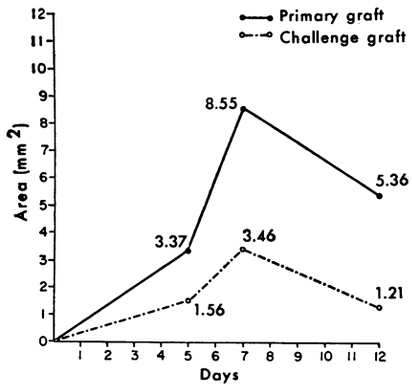

Fig. 1. The cross-sectional areas of the sites of the primary grafts were consistently larger than those of secondary grafts, the most striking differences occurring at day 7.

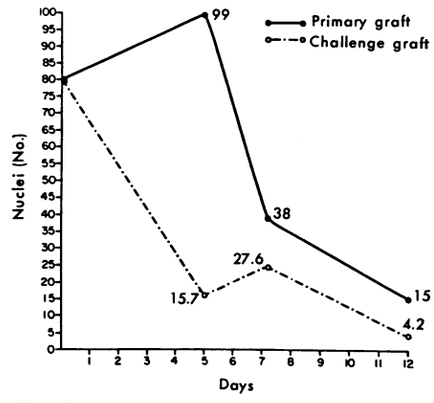

Fig. 2. Number of nuclei in trophoblast graft site. After 5 days of residence, trophoblast nuclei in primary grafts had increased in number whereas those in the challenge grafts had decreased. Trophoblast nuclei were consistently fewer in the challenge grafts throughout the period of growth.

Five days after transplantation, the recipient kidney was examined grossly. Hemorrhage at the site of transplantation invariably indicated trophoblastic growth. If this was found, the animal was retained for further studies. Groups of animals were killed 5, 7, and 12 days after transplantation. The kidneys were removed, and serial sections of the entire transplant sites were made for microscopic study.

Other groups of mice with successful grafts were exposed to a challenge graft of trophoblast tissue from 7½-day-old embryos syngeneic with the original grafts. These grafts were placed in the other kidney and were also inspected on the 5th day for growth. Groups of animals were again killed 5, 7, or 12 days after the second graft, and the various grafts were compared microscopically.

We performed 185 primary grafts. After the first 72 were done, the technique was sufficiently standardized that success rates of primary grafts could be compared with those of subsequent challenge grafts (Table 1).

Microscopic examination of sites of both primary and secondary grafts at 5 days revealed trophoblastic proliferation and destruction of capillaries at all but one graft site judged successful on the basis of subcapsular hemorrhage. We therefore accepted gross hemorrhage at 5 days as reliable evidence of tumor growth. A number of animals with successful primary grafts were killed for microscopic study. Animals with successful primary and challenge grafts were included for study if the scar of the old primary graft site could be demonstrated microscopically. With these restrictions, the number of animals qualifying for microscopic comparisons were: on day 5, ten with primary grafts and ten with challenge grafts; on day 7, eleven with primary grafts and eight with challenge grafts; on day 12, nine with primary grafts and ten with challenge grafts.

The area of the graft site including its accompanying hemorrhage was measured microscopically on the largest of the serial cross sections. These areas were averaged for each group (Fig. 1).

At the time of transplantation, a cross section of the ectoplacental cone transplanted contained about 80 nuclei. The cross sections of the subsequent graft sites containing the largest number of nuclei were selected for counting and averaging (Fig. 2). Figure 3 illustrates the histologic differences apparent between primary and challenge grafts.

We observed that, compared to the degree of invasion and metastasis to the medullary portions of the recipient kidney in primary grafts, active invasion of kidney tissue was less, and metastases were absent in challenge grafts. At 12 days, all but one of the primary graft sites still had trophoblastic nuclei, whereas only four of ten challenge grafts had them. Lymphocytic infiltration appeared in all stages of both primary and challenge grafts, but was slightly heavier in the latter. Cross sections of spleens removed when the ani-

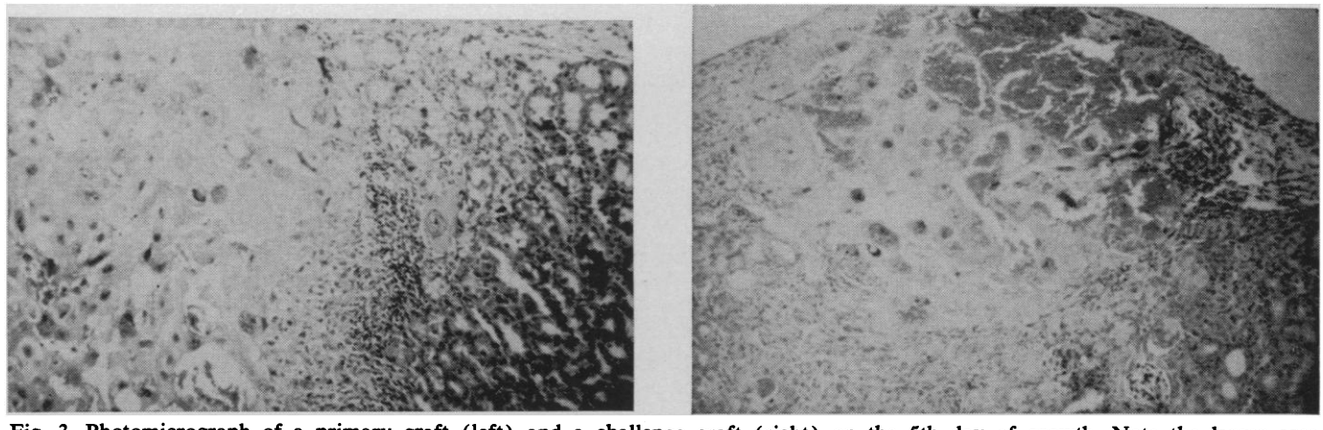

Fig. 3. Photomicrograph of a primary graft (left) and a challenge graft (right) on the 5th day of growth. Note the larger area of the trophoblast colony and the wandering invasive trophoblast in the primary graft compared to the more modest growth and containment of the colony in the challenge graft (× 400).

Table 1. Gross evidence of growth at 5 days in primary and challege grafts. Significance at $P \leq .05$ (by χ^2 method).

	Total (No.)	Growth (No.)	Rejection rate (%)
Primary	113	83	26.6
Challenge	35	19	46

mals were killed revealed depletion of lymphocytes in the Malphigian follicles to be most marked in animals 7 days after transplantation of challenge grafts. Spleens of these animals were larger as well.

The successful growth of these transplants was subject to a selective process partially affected by experimental technique and partially by the hosts' responses to the graft. With technique as a constant, the selective process eliminated almost twice as many challenge grafts as primary grafts (Table 1). Only the most vigorous challenge grafts were used for microscopic study. Therefore, the smaller values for these grafts (compared to primary grafts) in Figs. 1 and 2 are all the more noteworthy.

These studies indicate clear differences between primary and challenge grafts of trophoblast when judged by such criteria as gross success rate, size of the graft sites, number of viable cells at different stages, and host containment. The only difference between the test and control groups was that the test animals were previously exposed to a pure trophoblast colony which was absorbed. Therefore, prior exposure to trophoblast inhibits subsequent growth of this tissue, a phenomenon similar to the "second set" response demonstrating immunologic sensitization of a host to a prior graft.

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21 March 1968

Ruminant-Like Digestion of the Langur Monkey

Abstract. *The adaptation of langur monkeys to a laboratory environment has made possible a detailed investigation of their digestive physiology. The diverticular form of the langur stomach permits a bacterial fermentation of the leafy diet, which results in important contributions to the nutrition of these primates. The demonstration of a ruminant-like digestion in langurs extends the known taxonomic distribution of this digestive adaptation.*

Old World monkeys of the subfamily Colobinae differ from all other primates in the large size and anatomical complexity of the stomach. These differences are related to a diet consisting mainly of leaves, hence the name "leaf-eaters," commonly used to describe these primates. Members of the Colobinae are found in large numbers in a broad belt across Africa and throughout southern Asia. Those in the latter area are commonly known as langurs.

Gastric contents of the langur constitute a high proportion of the animal's body weight and are normally maintained at a *p*H of 5.0 to 6.7. This range of *p*H permits an active fermentation of ingesta by the large numbers of anaerobic bacteria present. Cellulose-digesting bacteria occur in high numbers. Fermentation products are volatile short-chain fatty acids, ammonia, carbon dioxide, methane, and small amounts of hydrogen. The acids are produced in sufficient quantities to make major contributions to the nutritional economy of the langur monkey.

Considerable literature has accumulated on the peculiarities of the gastric anatomy of the Colobinae (*1*). A superficial resemblance of the stomach to the rumen of herbivorous animals has been noted, but a number of authors (*2–4*) have stated that rumination does not occur. Although rumination is an obvious characteristic of ruminants, recent work has placed greater emphasis on the fermentative processes occurring in the rumen. A major question with regard to the Colobinae is whether such a microbial fermentation of ingesta does occur. Drawert *et al.* (*5*) analyzed samples of gastric contents obtained from colobus monkeys in Africa and found high concentrations of short-chain volatile fatty acids, similar in concentration and character to the fermentation end products found in rumen contents.

We experienced the universal problems in adapting members of the Colobinae to conditions of captivity (*6*). On arrival from Asian sources, *Presbytis entellus* and *Presbytis cristatus*, although seemingly starved, showed little interest in the various kinds of leafy and other foods obtainable commercially. Successful adaptation followed the discovery that fresh alfalfa, available locally for most of the year, was eaten eagerly by both species. When high intake of alfalfa was established, the diet could be expanded to include yams, green beans, and a cereal preparation, which were previously rejected.

The stomach of the silvered leaf monkey, *P. cristatus*, has features common to gastric apparatus of other Colobinae (*1, 7*). The greatly distended and sacculated portion (saccus gastricus), corresponding to the fundus, is followed by a tubular portion (tubus gastricus), which leads to a third or pyloric segment (Fig. 1). An examination of the internal structure in both species revealed an esophageal groove (canalis gastricus; *Magenstrasse*) which appears to be characteristic of ruminant-like animals (*8*). This structure may allow ingested liquids to pass directly from the esophagus to the middle compartment of the stomach (*7*).

With colobus monkeys, Kuhn (*2*) obtained values for gastric contents of 11.5 to 20.6 percent of total body weight. In a specimen of *P. cristatus*, with a terminal body weight of 5.4 kg, the gastric contents weighed 938 g, 17 percent of body weight. The *p*H of gastric contents withdrawn by stomach tube from 50 animals ranged from 5.0 to 6.7, permitting a bacterial fermentation of the leafy foods ingested. The large capacity of the stomach ensures a delayed flow of digesta essential for extensive fermentation.

High numbers of bacteria, but no characteristic protozoal flora, were found in gastric contents of both langurs. As determined with the anaerobic culture techniques of Hungate (*9*), viable anaerobic bacteria ranged from 7×10^{11} to 1×10^{12} per gram of dry matter. These counts were consistently higher than the values normally reported for rumen contents. The flora was complex, with the ratio of strict anaerobes to aerobes in the range 100:1 to 1000:1. As langurs thrive on a leafy diet it is significant that large numbers of cellulose-digesting bacteria (8×10^7 to 4×10^8 per gram dry matter) were present in gastric contents. Two forms were isolated, a Gram-