Since two similar ictal scans were obtained at different times on the same patient, these FDG patterns may be reproducible for each patient. Epileptiform discharges involving the temporal lobe resulted in increased metabolic activity in discrete limbic projection sites in only one patient (Fig. 1D). This diversity could indicate aberrations of normal functional anatomic connections as a result of, or in reaction to, the presence of a chronic focal epileptogenic lesion.

The FDG scan obtained after electroconvulsive shock therapy demonstrated that postictal depression is associated with a relative decrease in glucose metabolism. Consequently, the areas of relative hypometabolism seen on some ictal FDG scans during partial seizures may represent metabolic correlates of postictal phenomena. Ictal hypometabolism might also reflect surround inhibition (*14*). Although active inhibitory synaptic events appear to require energy (*15*), hypometabolism could occur at the efferent projection sites of inhibited neurons.

Two putative experimental models for clinical petit mal have been studied with 2DG autoradiography. Intracerebroventricular opioid peptides specifically activate limbic structures (*16*), whereas intramuscular penicillin fails to alter cerebral metabolism (Wada and Kobayashi, personal communication). The FDG scans of patients with petit mal revealed a reproducible diffuse increase in metabolic activity, suggesting that the pathological mechanisms for clinical and experimental petit mal are not the same. Although FDG scans failed to identify specific anatomic activation during petit mal seizures, a deep discrete generator responsible for initiation of the more generalized discharges could still be missed by this technique.

Patterns of ictal metabolic activity revealed by FDG scans from patients with partial and generalized seizures differ from patterns revealed by analogous 2DG autoradiographic studies of common animal models. Further studies are necessary to determine whether this represents true qualitative, or merely quantitative, differences between clinical and experimental epilepsy. It is necessary to bridge this gap between animal and human research in order to test hypotheses and develop clinically useful applications. Positron computed tomography, coupled with surface and depth electrode EEG recordings (*10, 11*), may provide a greater opportunity to determine which of the extensive electrophysiologic, metabolic, and anatomic data obtained from experimental models are relevant to human epileptic conditions and, ultimately, to pursue investigations into basic mechanisms of human epilepsy directly.

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Development of Mouse Embryos in vitro: Preimplantation to the Limb Bud Stage

Abstract. *Mouse embryos were grown successfully in vitro from the blastocyst stage to the limb bud stage. Mouse blastocysts grown in vitro for 10 days showed blood circulation in the yolk sac, forelimb buds, and the primordia of liver, pancreas, and lungs. These characteristics are indicative of a developmental stage equivalent to one-half of the total gestation period in utero. Improvements in culture conditions from days 7 to 9 have made it feasible to culture mouse blastocysts beyond the early somite stage.*

In the 1960's and early 1970's, investigators found means of culturing fertilized mouse ova in simple media up to the blastocyst stage (*1*). However, it has been difficult to grow mouse blastocysts in vitro beyond the egg-cylinder stage. Many investigators have cultured mouse embryos from the blastocyst stage to the egg-cylinder stage (*2*); few such blastocysts have reached the early somite stages (*3*) and none have survived beyond these stages. At the early somite stage, the blood circulation in the yolk sac is not established and the anterior neuropore is open. The limb buds and the primordia of the lung, liver, and pancreas are not yet present (*3*). We report here the successful culture of mouse embryos from the blastocyst stage to the limb bud stage.

Blastocysts (3.5 days of gestation) were obtained from random-bred CF1 mice (*4*) and cultured as described in Table 1. Of 86 blastocysts cultured for 10

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days, nine established yolk sac blood circulation (Fig. 1a). The forelimb buds of these embryos were clearly visible and the hind limb buds had begun to appear. The anterior neuropore was closed. The major divisions of the brain were apparent from the surface (Fig. 1b). The primordia of the lungs (Fig. 2a), liver, and dorsal pancreas were discernible. The optic vesicles (Fig. 2b) and the Wolffian ducts (Fig. 2d) were formed. The auditory vesicles were completely detached from the surface (Fig. 2c). These developmental characteristics indicate that the mouse blastocysts cultured for 10 days reached the developmental stage equivalent to 9.5 to 10 days of gestation in utero, or one-half of the total gestation period. That is, the external and internal morphology of 10-day embryos cultured in vitro is identical to that of 10-day embryos in vivo. Those cultured in vitro, however, were slightly larger than those gestated in utero. This might be due in part to the increased concentration of glucose added to the culture medium from days 7 to 10.

The heart of the cultured embryos began to beat on day 7 of culture. Blood cells in the vitelline vessels of the yolk sac did not circulate at this time but only oscillated with the heartbeats. Yolk sac blood circulation was first observed on day 8 of culture. Blood cells were not seen in the heart, aorta, or the cardinal veins (*5*) until the circulation occurred in the yolk sac (Fig. 1, a, c, and d). This indicates that yolk sac blood cells enter the heart and circulate within the embryo and the yolk sac only after the establishment of the vitelline vessels between the yolk sac and the embryo proper.

We attribute the successful growth of mouse embryos in vitro from the blastocyst stage to the limb bud stage to certain improvements in the culture conditions from days 7 to 9: (i) use of rat serum during later stages (*6*); (ii) continuous agitation of the culture medium and embryos from days 7 to 10; and (iii) an increase in the proportion of oxygen in the gas mixture. Among three types of serum used in embryo culture (fetal calf serum, human cord serum, and rat se-

Table 1. Procedures for culturing mouse embryos. Two blastocysts, after 3.5 days of gestation, were grown in a 35-mm Falcon plastic culture dish containing 2 ml of culture medium. The basic medium was CMRL 1066, which was supplemented with 1 m*M* glutamine, 1 m*M* sodium pyruvate, and serum (*6*). The concentration and type of serum used varied with days in culture. The culture medium was changed daily except day 10. The cultures were maintained at 38°C in humidified incubators containing 5 percent CO_2 and various proportions of O_2. The culture dishes were kept stationary from day 0 to day 7 and were agitated continuously on a shaker at 60 oscillations per minute from day 7 to day 10. No antibiotics were used during culture.

Day of culture	Serum concentration (%)			Oxygen concentration (%)	Physical treatment of culture dish
	Fetal calf serum	Human cord serum	Rat serum*		
0	10			20	Stationary
1	10			20	Stationary
2	20			20	Stationary
3	10	10		20	Stationary
4		30		20	Stationary
5		40		20	Stationary
6		50	10	20	Stationary
7			100	5	Shaking
8			100	20	Shaking
9			100	40	Shaking
10					Shaking

*Plus 0.3 percent glucose.

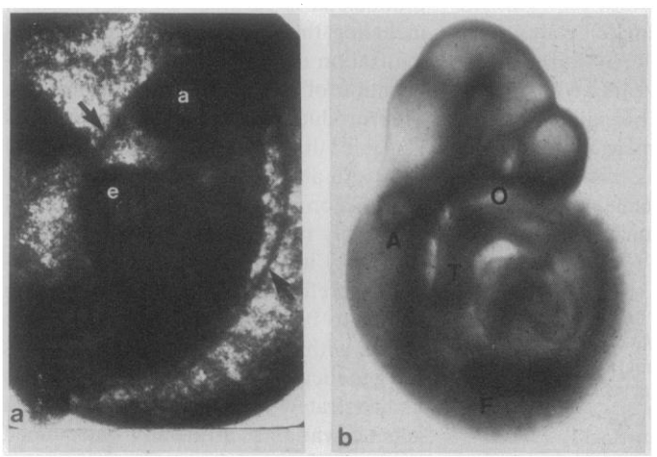

Fig. 1 (left). (a) A dorsal view of a live mouse embryo that was removed during the blastocyst stage (3.5 days of gestation) and cultured for 9 days and whose yolk sac blood cells had just begun to circulate. The yolk sac blood circulation was recorded with a Kodak Ektachrome 16-mm movie film (7242) from which this photograph was obtained. The rotation of the embryo had taken place. The embryo was enclosed by the amnion, which is indicated by the arrows. Both the embryo and amnion were contained within the yolk sac. (*a*, brain; *e*, heart) (×25). (b) A mouse embryo that was removed during the blastocyst stage (3.5 days of gestation) and cultured for 10 days. The yolk sac and amnion were removed and the whole embryo was fixed in glutaraldehyde. Unstained (*A*, auditory vesicle; *F*, forelimb bud; *O*, optic vesicle; *T*, tail). (×30).

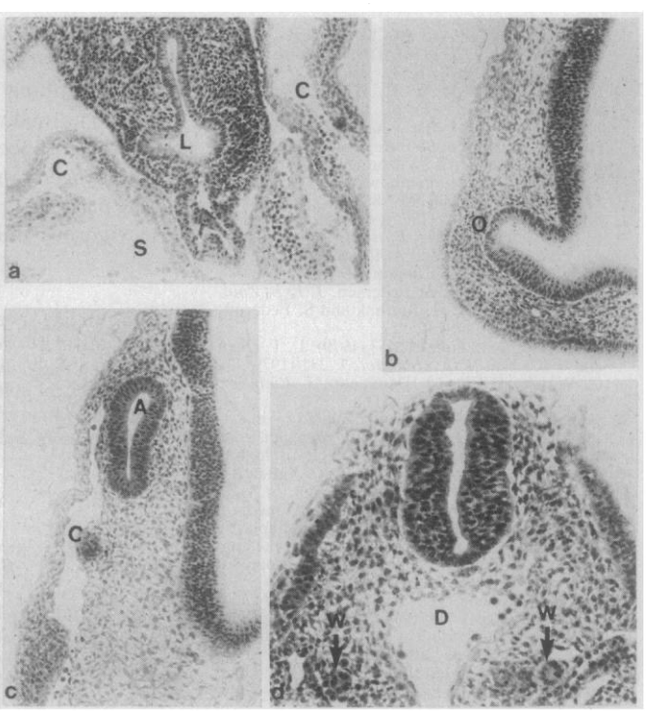

Fig. 2 (right). Lung bud, optic vesicle, auditory vesicle, and Wolffian duct in mouse embryos that were removed during the blastocyst stage (3.5 days of gestation) and cultured for 10 days. Blood cells were present in vascular spaces. (a) *L*, Lung bud; *C*, common cardinal vein; *S*, sinus venosus. (b) *O*, Optic vesicle. (c) *A*, Auditory vesicle; *C*, anterior cardinal vein. (d) *D*, Dorsal aorta; *W*, Wolffian duct. The methacrylate sections (3 μm) were stained with hematoxylin and eosin. (a to c) ×60; (d) ×120.

rum), rat serum was found to be best from days 7 to 9. Although rat serum could be used prior to day 7 to replace fetal calf serum and human cord serum, the success rate of culturing mouse blastocysts to the limb bud stage decreased (*7*). The reason for this is unknown. Rat serum is an excellent culture medium for explanted early somite rat embryos (*8*) as well as mouse embryos (*9*). Continuous agitation of the culture medium and embryos may have greatly facilitated the exchange of nutrients, metabolites, and the gases between the embryo and the medium. On day 8 of culture, the embryos rapidly expanded in size and began to establish the blood circulation. A higher percentage of oxygen in the incubator at this stage appeared to be beneficial.

Although mouse embryos can be grown continuously in culture from the blastocyst stage to the limb bud stage, the success rate remains low. A higher success rate will require further improvements of the culture conditions.

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5. Whole mouse embryos were fixed in 0.1*M* cacodylate buffer (pH 7.2) containing 4 percent glutaraldehyde for 2 hours at room temperature and washed in the same buffer for 2 hours. Embryos were then dehydrated in ethanol and embedded in butoxylethanol glycol methacrylate (Polysciences, Warrington, Pa.). Sections 3 μm in thickness were stained with hematoxylin and eosin.
6. Fetal calf serum was obtained from Gibco; human cord serum was prepared from the umbilical cord blood [Y. C. Hsu, *Dev. Biol.* **68**, 453 (1979)], and rat serum was prepared from blood centrifuged immediately after extraction from the rat [C. E. Steele and D. A. New, *J. Embryol. Exp. Morphol.* **31**, 707 (1974)].
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Decay of Female Sexual Behavior Under Parthenogenesis

Abstract. *A laboratory strain of* Drosophila mercatorum *has existed for 20 years without males and therefore without natural selection operating to maintain the genetic basis of female mating behavior. The females of this strain have recently experienced a genetic impairment of mating capacity. This observation exemplifies the mode of evolution of vestigial characters and supports Muller's theory that random mutation will tend to destroy the genetic basis of a character from which selection has been removed.*

Thirty years ago, Muller (*1*) proposed that random mutations would destroy the genetic basis of a character unless natural selection functioned to maintain its integrity. According to the concept, if selection is removed, a character will decay down to the level at which some kind of positive natural selection becomes reimposed. This hypothesis provides a simple genetic explanation for such curious biological facts as the decline of wings to vestiges in flightless birds and the disappearance of eyes in cave animals. Evidence for such an apparently fundamental principle of genetic change in evolution is virtually lacking. Microevolutionary data that directly support Muller's prediction are presented here.

Unisexual (all female) laboratory strains of *Drosophila mercatorum* were established about 20 years ago by selection from normal wild bisexual ancestors (*2*). In the absence of male courtship (the selective agent in this case), a loss of mating propensity in females of an otherwise vigorous unisexual strain was observed. This loss is genetically based and is permanent.

Table 1. Mating speed of females from laboratory strains of *Drosophila mercatorum*. Control bisexual Salvador females (sets A, B, and C) and a control unisexual strain K-23 (sets D and E) are compared with strain S-1, a unisexual derivative of Salvador stock.

Data set	Strain	Year	Females tested (No.)	Mating speed*
		Bisexual		
A	Salvador	1973	167	90.4
B	Salvador	1980	87	84.0
C	Salvador	1981	149	81.9
		Unisexual		
D	K-23	1973	99	80.8
E	K-23	1981	150	74.6
F	S-1	1973	150	85.3
G	S-1	1980	90	20.0
H	S-1	1981	150	30.0
I	S-1-T	1981	160	18.1

*Percent mated in 30 minutes.

The unisexual laboratory strains that we used were established by isolating virgin females from bisexual laboratory stocks and by selecting for the enhancement of a naturally present, low-grade facultative parthenogenesis. Most strains produce occasional sterile males, but these usually number less than 0.5 percent. One strain in particular (S-1) has been maintained in the laboratory by routine culture since July 1961 when it was originally established (*2*, *3*).

All females in these strains are diploid; most oviposit well and produce daughters that are diploid like their mothers. Meiosis occurs normally and a haploid pronucleus is formed. In most strains, including the ones described here, diploidization occurs predominantly by duplication of this single haploid nucleus. This produces a diploid female that is isogenic, that is, homozygous at all loci (*4*–*6*). An alternative mode of diploidization, fusion between the pronucleus and a possibly nonidentical polar nucleus, is ordinarily infrequent. Soon after founding, therefore, each laboratory strain comes to be composed of one or more clones within each of which the females are genetically identical. This effect was demonstrated in experiments in which genetic variants artificially introduced into a stock were observed to survive as a series of coexisting parallel clones for a number of parthenogenetic generations (*7*). Some clones survived longer than others.

Sexual behavior in this species is influenced by an extensive polygenic basis. This has been demonstrated by artificial selection experiments (*8*) and by crosses between bisexual strains (*9*). Among a number of the unisexual strains produced in the laboratory, the mating propensity of the females is not different from that displayed by the ancestral bi-

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