the trial was terminated and the next trial began 40 seconds later. Beginning the next day, all animals were subjected to five trials per day until there were two consecutive days in which no shock was received, or 10 successful avoidance trials in two days (criterion).

The data were analyzed by the procedures (*8*) for analysis of variance for a 2 by 2 factorial design with unequal numbers in each cell. Scores of the trials through criterion and the sum of the median latencies for the first four days were used to assess the effects of infant handling on acquisition of avoidance conditioning. The experimental group was composed of four males and six females. There were five males and 12 females in the control group. There were no significant differences between the handled and nonhandled groups or between the males and females, nor were there significant interactions on either measure.

These results, not in agreement with others (*2*), are probably attributable to differences in apparatus. Where infant handling facilitated avoidance learning the animals were required to open an escape door in order to leave the shock compartment. In our experiment, the animal learned only the simple response of running from one compartment to the other at the onset of the buzzer. Criterion was reached by 50 percent of the animals in 30 trials; the average for all animals was six errors during the first 20 trials. The escape door in the previous experiments may have inhibited responding by the more emotional nonhandled animals and thus differentially have affected the rate of acquisition. This explanation is substantiated by Levine's (*2*) observation that the nonhandled animals take a greater number of trials before making their first avoidance response.

The means of the brain weights and cholinesterase activity for the handled and nonhandled animals are presented in Table 1. These data are based on the six males and four female animals from the handled group and seven males and 14 females from the nonhandled group. The statistical analysis of the data indicated that sex was a significant main effect for the subcortex and dorsal-cortex weights and dorsal-cortex cholinesterase activity; the males showed higher weights and lower cholinesterase activity. The handling procedure produced significant increases in the weights of the subcortex and the ventral cortex and significant decreases in cholinesterase activity in the subcortex.

The results of the analysis of the brain weights and cholinesterase activity are more easily interpreted when considered with the experiments of Krech *et al.* (*5*) in which postweaning experience was manipulated. There, animals raised in enriched environments show greater weight and lower cholinesterase activity in the cortex than littermates raised in isolation. Subcortical cholinesterase tends to be higher for the enriched animals and there are no differences in subcortical weights.

The results of our experiment are in direct contrast to those of Krech *et al.* (*5*). The measures on the cortex do not differ between the handled and nonhandled groups but the subcortical measures do. These results imply that pre- and postweaning experiences produce different effects on brain weight and brain cholinesterase; the infant handling procedures affect the subcortical brain while postweaning environmental enrichment affects the neocortex.

The specific mechanisms which cause these alterations in brain weight and cholinesterase are unknown. These results do suggest that the behavioral changes associated with the infant handling and environmental enrichment procedures may be mediated by changes in the central nervous system that affect central cholinergic mechanisms (*9*).

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Mitoses: Distribution in Mouse Ear Epidermis

Abstract. *Epidermal cell mitoses occur in a random spatial (Poisson) distribution in the plane of the epidermis of the mouse. It is therefore unlikely that the focal accumulation of a local extracellular factor is responsible for the initiation of mitoses.*

When mitoses are counted in a tissue such as the epidermis, they sometimes seem to occur in clusters (*1*). This may be of some importance, for if mitoses indeed occur in clusters, it would suggest that some focal factor which acted on a group of cells was concerned in the initiation of cell division.

In order to test this hypothesis one ear of each of five anesthetized adult, Swiss male mice was removed with sharp scissors, and a 3 by 4 mm rectangle was then cut from the excised ear. These biopsies were placed immediately in 0.5 percent acetic acid at 4°C and incubated for 5 hours to allow separation of the epidermis from the dermis. The epidermis was then dissected off with a spear-point needle, stretched with straight pins on a piece of cork and fixed in 10 percent formalin overnight (*2*). The tissue was stained with haemalum (*3*), dehydrated in three changes of absolute alcohol, cleared in three changes of toluol, and mounted with the basal cell layer up. Such specimens, when examined with an oil-immersion lens, show epidermal cells and their mitoses clearly.

The preparations consist almost entirely of a single layer of basal cells covered by a very attenuated layer of flattened squamous cells. This two-dimensional tissue is well suited to a study of the distribution of mitoses. The proportion of cells in mitosis was determined in each specimen by means of an ocular grid in a square area of 100 grid units, each unit containing an average of 700 to 800 nuclei. Because of Gelfant's (*4*) observation that there is an increased mitotic frequency near the cut edge in such biopsies, only the areas distant from the cut edges were counted, even though the immediate immersion of the biopsies into cold acetic acid may have prevented this phenomenon.

Mitoses occurring in hair follicles were ignored. Hair follicles made up less than one-eighth of the area surveyed, and the space they occupied was considered as not containing mitoses.

Table 1. Calculation of Poisson fit for the distribution of mitoses in sample No. 1. (Chi-square, 6.26; degrees of freedom, 6; probability level, 0.30 to 0.50.)

Mitoses	Grid unit (No.)*		$O-E$*	$\frac{(O-E)^2}{E}$
	Observed (O)	Expected (E)		
0	1	1.5	−1.8	0.42
1	5	6.3		
2	16	13.2	+2.8	0.59
3	17	18.5	−1.5	0.12
4	26	19.4	+6.6	2.25
5	11	16.3	−5.3	1.72
6	9	11.4	−2.4	0.51
7	9	6.9	+2.1	0.64
8	2	3.6	−0.3	0.01
9	1	1.7		
10	2	0.7		
11	1	0.3		
12	0	0.0		

* Pooled where number of grid units was less than 5.

Our impression was that the hair-follicles were distributed evenly throughout the samples but their density was too low to allow statistical analysis.

The data we obtained on mitoses were analyzed. Since the mitotic frequency in all specimens was less than 0.5 percent, the assumption was made that should mitoses be distributed randomly, their distribution would be akin to the Poisson distribution. If the mean number of mitoses per grid unit is designated λ, then according to the Poisson distribution the probability that a grid unit contained m mitoses is $e^{-\lambda}(\lambda^m/m')$. With this formula, in which e is the base of natural logarithms, the expected number of grid units containing a given number of mitoses was calculated and the results were compared with the observed values. The chi-square criterion was used for the testing of goodness of fit. Using the observed values (O) and the expected values (E) the chi-square value of $\Sigma(O-E)^2/E$ was calculated for each specimen and evaluated by standard chi-square tables. Table 1 shows the calculations for sample No. 1. Table 2 shows the findings in all samples.

Table 2. Distribution of mitoses in mouse ear epidermis compared to Poisson distribution.

Total mitoses observed	χ^2	df*	P level
	Ear sample 1		
420	6.28	6	0.30–0.50
	Ear sample 2		
171	2.79	3	0.30–0.50
	Ear sample 3		
408	12.43	6	0.05
	Ear sample 4		
241	1.84	4	0.75
	Ear sample 5		
199	3.57	4	0.50

* Degrees of freedom.

In all five mice the probability that the distribution of mitoses in the basal cell layer of the ear epidermis differed by chance alone from the Poisson distribution was 0.05 or greater. This method of analysis has thus brought forth no evidence that mitoses in mouse ear epidermis occur in clusters.

Our results are in agreement with the observations of Meyer, Medak, and Weinmann on the epithelium of the palate of mice (*5*) and those of Brues and Marble on the regenerating rat liver (*6*). Both of these groups found mitoses present in a Poisson distribution.

However, Harkness (*7*) who also studied mitoses in regenerating rat liver, warned that the grid unit used must not be too small. In his experiments mitoses appeared to be in a Poisson distribution when a small grid unit containing about 30 nuclei was used, but not with a larger grid unit containing about 270 nuclei. However, the grid unit we used was even larger, and the epidermis is a more homogeneous tissue than the liver.

There is no doubt that when the entire body surface is considered, there are wide variations of epidermal mitotic activity (*1*), but as far as a single location is concerned, the results of our experiment do not support the theory that mitoses are, at least in part, triggered by a local accumulation of a cell product. This does not necessarily mean that some general humoral or other mechanism is concerned [although this possibility has some experimental support (*8*)], since the observed chance distribution of mitoses is also compatible with the theory that cell division is stimulated by uniformly distributed intracellular needs.

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Dating Skeletal Material

Abstract. *A nitrogen test has become ancillary to radiocarbon dating.*

A system of relative dating of fossil bone, antler, and dentine has been developed during recent years (*1*). It combines fluorine analysis with uranium estimation by radiometric assay and nitrogen determination by a micro-Kjeldahl method. As a by-product of this research, estimation of the nitrogen content of fossil bone, antler, and dentine is now being used in Britain as a convenient means of assessing the organic carbon content of these materials, since the nitrogen content is an index of the residual collagen (the C/N ratio is about 2.5). Thus the nitrogen test has become ancillary to radiocarbon dating of bone and other skeletal material.

The recent dating of the Galley Hill skeleton (*2*) serves to illustrate the procedure. Analysis of suitably washed powder drilled from one of the humeri of the skeleton showed 2.0 percent nitrogen. It was estimated that this bone therefore contained about 5 percent collagenous carbon. Casts were made of both humeri, and portions weighing about 30 grams were cut off and pulverized. The powder was subjected to repeated washing in warm water and then in acetone to remove any possible traces of preservatives (*3*). The radioactivity was then measured in the Radiocarbon Laboratory of the British Museum and the results gave a radiocarbon age of 3310 ± 150 years (*4*). This confirmed the relative dating by the fluorine method (*2*), indicating that the skeleton was probably a post-Pleistocene intrusive burial.

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