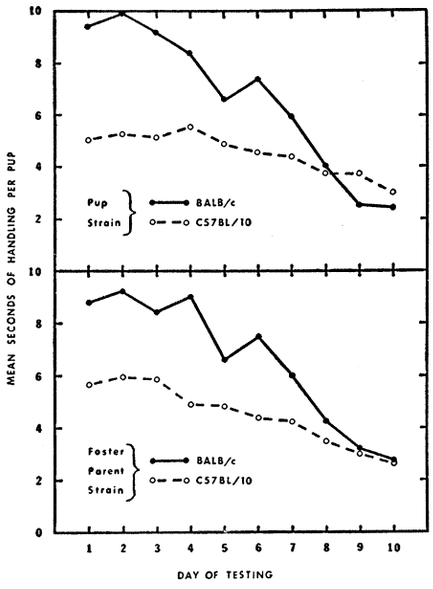


Fig. 1. Mean number of seconds of handling per pup of the two strains of pups (top) and by the two strains of parents (bottom) as functions of day of testing.

mental variable is of particular importance in an attempt to assess the purely genetic influences upon behavior.

In order to evaluate differences in handling due to the strain of the parents independently of differences in handling due to the strain of the pups, a foster rearing scheme was employed. Ten litters of the C57BL/10 strain were reared by foster parents of their own strain and ten litters were reared by foster parents of the BALB/c strain. Similarly, ten litters of the BALB/c strain were reared by foster parents of their own strain and ten litters were reared by foster parents of the C57BL/10 strain. Of the 40 litters, 33 were switched to foster parents on the day they were born and no litter was switched at more than 4 days of age. All animals were housed in aluminum pans (9⅝ by 5½ by 2¾ in.) with cedar shavings as a nesting material.

Table 1. Analysis of variance of the mean number of seconds of handling received per pup.

| Source | df | MS | F |
|---|---|---|---|
| Between litters | 39 | | |
| Pup strain ($P$) | 1 | 421.83 | 9.38* |
| Foster parent strain ($F$) | 1 | 439.72 | 9.78* |
| $P \times F$ | 1 | 10.43 | .23 |
| Error (between) | 36 | 44.97 | |
| Within litters | 360 | | |
| Days ($D$) | 9 | 128.55 | 14.98† |
| $D \times P$ | 9 | 42.39 | 4.94† |
| $D \times F$ | 9 | 19.15 | 2.23‡ |
| $D \times P \times F$ | 9 | 11.56 | 1.35 |
| Error (within) | 324 | 8.58 | |

* $p < .005$. † $p < .001$. ‡ $p < .05$.

The measurement of parental handling began on the day after the litters were switched and continued for 10 successive days. On each test day the foster parents were removed from the nesting cage and placed in an empty cage nearby. The pups were taken from the nest and placed in the opposite end of the nesting cage. The foster parents were then returned to the nesting cage. During the succeeding 5 minutes the total number of seconds of handling of the pups by the foster parents was recorded on an electric timer which was activated by the experimenter. Handling was defined as the carrying, dragging, or oral manipulation of a pup by either of the foster parents.

The total number of seconds of handling recorded for a litter on each day was divided by the number of pups in the litter to yield a measure of the average amount of handling received per pup during each test session. The interobserver reliability of this measure for a sample of 50 sessions was found to be .98 by product-moment correlation.

Table 1 presents an analysis of variance of the average amount of handling received per pup. Significant effects were found to be due to the strain of the pups and to the strain of the foster parents. The BALB/c parents handled both strains of pups more than the C57BL/10 parents did, and the BALB/c pups received more handling from both strains of parents than the C57BL/10 pups did. In addition, the amount of handling showed a significant decline with successive days of testing ($D$), and the magnitude of the effects of pup strain ($P$) and foster-parent strain ($F$) decreased over the course of the 10-day period, as reflected by the significant $D \times P$ and $D \times F$ interactions. The effects of pup strain and foster-parent strain as functions of day of testing are shown in Fig. 1.

These results indicate that there is a difference in the amount of handling received by two strains of mice during infancy. The difference in parental handling due to the strain of the pups cannot be regarded as stemming from a genetic difference between the two strains of parents. Rather, the strain of the offspring must be considered an environmental variable for the parents whose behavior was being measured. In addition, the difference in the parental behavior of the two strains (irrespective of the strain of the pups) may itself be due not to a genetic difference but to a difference in the way the parents were handled as infants.

In general, behavioral differences between strains observed in adulthood should not necessarily be attributed to genetic rather than early environmental variation (*3*).

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21 March 1962

## Tracers, Transfer through Membranes, and Coefficients of Transfer

*Abstract.* The rate of flow of a tagged species of a material substance through a permeable membrane is proportional to the rate of flow of the substance itself when, and only when, the species mole fraction of the substance is the same on both sides of the barrier. The ratio of the osmotic transfer coefficient of a substance in a particular barrier to the exchange coefficient, determinable with a tracer, is greater than 1.

The relation between the rate of flow of a substance $\dot{n}_s$ and the rate of flow of a tagged species of the substance, $\dot{n}_{s'}$, across a plane of observation located within the barrier of the linear-transfer system phase $\alpha$, membrane, phase $\beta$ has been derived (*1*). A more general but less awkward derivation of the same equation is given later in this report (*2*).

The general equation for the rate of flow of $s$ across a plane of observation may be written

$$-\dot{n}_s = \sum_k M_{sk} \frac{d\mu_k}{dx} \qquad (1)$$

Here the $M_{sk}$'s are the generalized admittance coefficients of a barrier and $d\mu_k/dx$ is the algebraic representation

of the chemical potential gradient of a neutral molecule, or the electrochemical potential gradient of an ion in the barrier of a linear transfer system. The summation is to include all the kinetic species present at the plane of observation (*3*). An outline of the derivation of Eq. 1 from Newton's first and second laws of motion applied to steady-state transfer systems, and a demonstration of the validity of the Onsager reciprocal relations $M_{sk} = M_{ks}$, based upon Newton's third law of motion, have also been presented (*4*).

Suppose the transfer system to be closed to $s$. Imagine that the substance $s$ is composed of two subspecies, $s'$ and $s''$, whose kinetic properties are identical. Let $s'$ and $s''$ have some property by which they can be distinguished but which has no effect on their motions. The species mole fraction of the two subspecies and other relations among them can be written as follows:

$$\begin{aligned} n_{s'} &= N_1 n_s, \quad \text{, also } N_1 + N_2 = 1 \\ \dot{n}_{s'} &= N_1 \dot{n}_s + n_s \dot{N}_1 \qquad dN_1 = -dN_2 \end{aligned} \tag{2}$$

The equations for the flow rates of the two subspecies across the plane of observation may be obtained by writing Eq. 1 in greater detail. They are

$$\begin{aligned} -\dot{n}_{s'} &= M_{11}\frac{d\mu_1}{dx} + M_{12}\frac{d\mu_2}{dx} + \sum_{k=3}^{s} M_{1k}\frac{d\mu_k}{dx} \\ -\dot{n}_{s''} &= M_{21}\frac{d\mu_2}{dx} + M_{22}\frac{d\mu_2}{dx} + \sum_{k=3}^{s} M_{2k}\frac{d\mu_k}{dx} \end{aligned} \tag{3}$$

The flow rate of the substance $s$ is the sum of the flow rates of the two subspecies, or

$$-\dot{n}_s = (M_{11} + M_{12})\frac{d\mu_1}{dx} + (M_{12} + M_{22})\frac{d\mu_2}{dx} + \sum_{k=3}^{s}(M_{1k} + M_{2k})\frac{d\mu_k}{dx} \tag{4}$$

when use is made of the Onsager reciprocal relations. Let $d\mu_1/dx$ and $d\mu_2/dx$ in Eqs. 3 and 4 be zero. The following equations then hold:

$$-\dot{n}_1 = -N_1\dot{n}_s = N_1 \sum_{k=3}^{s}(M_{1k} + M_{2k})\frac{d\mu_k}{dx} = \sum_{k=3}^{s} N_1 M_{sk}\frac{d\mu_k}{dx} = \sum_{k=3}^{s} M_{1k}\frac{d\mu_k}{dx} \tag{5}$$

since the $(d\mu_k/dx)$'s cannot produce an $\dot{N}_1$ or an $\dot{N}_2$ because of the presumed identical kinetic properties of $s'$ and $s''$. Eqs. 5, therefore, require that

$$M_{1k} = N_1 M_{sk} \text{ also } \frac{M_{1k}}{M_{2k}} = \frac{N_1}{N_2} \tag{6}$$

The relations of the chemical potential gradients of the two subspecies to the chemical potential gradient of the species $s$ and the gradient of mole fraction are known (*1*). These may be written

$$\begin{aligned} \frac{d\mu_1}{dx} &= \frac{d\mu_s}{dx} + RT\frac{d\ln N_1}{dx} = \frac{d\mu_s}{dx} + \frac{RT}{N_1}\frac{dN_1}{dx} \\ \frac{d\mu_2}{dx} &= \frac{d\mu_s}{dx} + RT\frac{d\ln N_2}{dx} = \frac{d\mu_s}{dx} - \frac{RT}{N_2}\frac{dN_1}{dx} \end{aligned} \tag{7}$$

Replacing the chemical potential gradients in Eq. 4 by their equivalents, as given by Eq. 7, yields

$$-\dot{n}_s = (M_{11} + 2M_{12} + M_{22})\frac{d\mu_s}{dx} + \left[M_{11} + M_{12} - \frac{N_1}{N_2}(M_{12} + M_{22})\right]RT\frac{d\ln N_1}{dx} + \sum_{k=3}^{s} M_{sk}\frac{d\mu_k}{dx} \tag{8}$$

Now the gradient, $d\ln N_1/dx$, cannot affect $\dot{n}_s$ since $n_{s'}$ and $n_{s''}$ are presumed to have identical kinetic properties. Thus, in order for Eq. 8 to be exact, the following relation must hold.

$$M_{11} + M_{12} = \frac{N_1}{N_2}(M_{12} + M_{22}) \tag{9}$$

and $\dot{n}_s$ is given by

$$-\dot{n}_s = (M_{11} + 2M_{12} + M_{22})\frac{d\mu_s}{dx} + \sum_{k=3}^{s} M_{sk}\frac{d\mu_k}{dx} \tag{10}$$

By substituting the relations given in Eqs. 7 back into the first member of Eqs. 3 and using the relations given by Eqs. 6 and 9, we may now write $\dot{n}_{s'}$ as

$$-\dot{n}_{s'} = N_1\left[(M_{11} + 2M_{12} + M_{22})\frac{d\mu_s}{dx} + \sum_{k=3}^{s} M_{sk}\frac{d\mu_k}{dx}\right] + \left(\frac{M_{11}M_{22} - M_{12}^2}{M_{12} + M_{22}}\right)\frac{RT\,d\ln N_1}{dx} \tag{11}$$

On comparing this equation with Eq. 10 it may be seen that

$$-\dot{n}_{s'} = -N_1\dot{n}_s + M_{ex}RT\frac{d\ln N_1}{dx} \tag{12}$$

Here $M_{ex}$, designated as an exchange coefficient, is taken as equal to

$$(M_{11}M_{22} - M_{12}^2)/(M_{12} + M_{22}).$$

Equation 12 is the basic equation for the relation of the rate of flow of a tagged species across a plane of observation to the rate of flow of the species itself, and it may be applied to an actual experiment if the tagged subspecies does not differ materially in molecular mass or density from the second subspecies. In the majority of biological experiments the errors introduced by assuming identical kinetic properties for two subspecies containing isotopes of the same chemical element are usually much smaller than the experimental errors; therefore, neglecting isotope effects is entirely proper. As Eq. 12 implies, the flow of the tagged material is proportional to the flow of the substance itself when, and only when, the mole fraction of the species is the same in the two phases adjacent to the barrier.

In the usual tracer experiments the tracer is added to one phase or the other in "weightless" amounts when radioactive isotopes are used as tags, or in small amounts when stable isotopes are employed. This means that $N_1$, the species mole fraction of the tracer at the plane of observation, approaches zero, and that the observed flow of the tracer bears little relation to the flow of the substance concerned. Such was the case, for example, in the experiments performed by Ussing (*5*) and by Durbin, Frank, and Solomon (*6*). In the experiments of Prescott and Zeuthen (*7*), $N_1$ was appreciable, and then the flow of the substance concerned did have a measurable effect on the flow of tracer. Most tracer experiments allow one to determine only the magnitude of $M_{ex}$ for a particular substance in a particular membrane.

If separate experiments of the proper kind are carried out on a particular membrane, the ratio $M_{os}/M_{ex}$, where $M_{os}$ is defined as equal to $M_{11} + 2M_{12} + M_{22}$ (see Eq. 10) should be greater than 1, for

$$\frac{M_{os}}{M_{ex}} = \frac{(M_{11} + 2M_{12} + M_{22})(M_{12} + M_{22})}{M_{11}M_{22} - M_{12}^2} > 1 \tag{13}$$

since $M_{11}M_{22} - M_{12}^2$ is positive. The experiments of Hevesy, Hofer, and Krogh (*8*) on the frog's skin yielded "unexplained" ratios of 3 to 5, and the ratios obtained for certain artificial membranes by Mauro (*9*) were several hundred times these values.

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8 March 1962

## High-Speed Cinematography of Muscle Contraction

*Abstract.* Motion pictures of the "twitch" of an excised frog gastrocnemius muscle taken at rates of 6000 frames per second provide a means of very accurately timing the phases. The extreme "slow motion" reveals surface phenomena not observable by other techniques. Evidence of "active relaxation" is suggested by results of frame-by-frame analysis.

High-speed cinematography (frame rates in excess of 500 per second) has had wide application in researches in the fields of engineering and technology. Very limited application has been made to the study of rapid biological phenomena. Farnsworth *et al.* (*1*), Prinzmetal *et al.* (*2*), and Zorgniotti *et al.* (*3*) have strikingly demonstrated the value of the technique in the analysis of various motion phenomena in human subjects.

The phases of a simple muscle twitch (latency, contraction, relaxation) have been accurately timed by various electronic and photographic techniques. None of these, however, has provided the experimenter with a continuous pictorial representation of the muscle during the events.

The excised muscle preparation, illuminated with incandescent light (24,000 ft-ca) was photographed with a "Fastax" camera operating at 6000 frames per second (*4*). The motion pictures were studied by projection at normal (24 frames per second) speed and were also subjected to frame-by-frame analysis. The muscle was stimulated to contract by a single shock from an electronic stimulator, and an electromagnetic signal marker was included in the camera field to indicate the instant of stimulation. The muscle was loaded with a shortened Harvard-type muscle lever and a 10-g weight. The muscle was marked by a "tattooing" technique. This consisted of placing a series of black dots at intervals on the surface of the muscle to provide distinct reference points in the frame-by-frame analysis. The muscle preparation was photographed against a grid background.

Direct observation of the projected motion pictures reveals several interesting features. It may be easily seen and proved that the mass inertia of the lever system causes the lever movement to lag considerably behind the movement of the dots on the muscle except during the latter portion of the relaxation phase. Frame-by-frame analysis of a typical twitch provides data regarding the duration of the phases as indicated by the movement of the muscle dots and by the movement of the lever system (Table 1).

Careful observation of the projected film shows certain expected changes in the diameter of the muscle during contraction and relaxation. These are not, however, as simple in form as one might have expected. They appear as bulges traveling downward during contraction and upward during relaxation. Their movement is extremely rapid. Attempts to ascertain the velocity of these contraction and relaxation waves by frame-by-frame analysis were not considered valid because of the difficulty of exactly tracing and measuring the muscle image from projected single frames. This difficulty is due to an inherent lack of image definition in many high-speed motion pictures.

Both by simple viewing of the projected film and by the frame-by-frame analysis it is readily shown that the initial portion of the relaxation phase is extremely rapid. A plot of the movement of the tattoo marks on the muscle shows this part of the relaxation phase to be as rapid as, if not more rapid than, any portion of the contraction phase. No definite physiological conclusions are drawn from this, but it suggests an "active" type of relaxation. Later portions of the relaxation phase were slow by comparison, and the muscle did not return to its fully relaxed state until the lever system had caught up with it in time. Exact true relaxation time, therefore, could not be determined.

Table 1. Duration of phases of a simple muscle twitch of an excised frog gastrocnemius as shown by frame-by-frame analysis of high-speed (6000 frames per second) motion pictures.

| Latency (sec) | Contraction (sec) | Relaxation (sec) |
|---|---|---|
| *Movement of dots (true time)* | | |
| 0.003 | 0.037 | ? |
| *Movement of lever (apparent time)* | | |
| 0.007 | 0.044 | 0.052 |

Extreme slow-motion projection of the film suggests one further phenomena which is noteworthy but unsubstantiated by concrete evidence. There appears to be an undulatory movement over the surface of the muscle during both its contraction and its relaxation. This phenomenon, if it actually occurs, is extremely rapid and not at all distinct. It is suggested that it could be a high-frequency surface vibratory movement and, if this is the case, might be a purely physical result of tension developed in an elastic system rather than anything of physiological significance.

The data giving accurate time relationships in the simple muscle twitch, as well as the surface phenomena observed when action is slowed by a factor of 250, clearly indicate the applicability of high-speed cinematography to the study of muscle activity. Further experiments of this type should not only improve the techniques but provide data of important physiological significance (*5*).

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