made to prevent the birds from responding in the presence of the negative stimulus. In fact, to insure that all birds in this group made errors, each animal was given 2 days of continuous reinforcement in the presence of both the 555-nm light (which became the positive stimulus) and the white vertical line (which became the negative stimulus). After key-peck training, the reinforcement schedule was changed to a variable-interval schedule with a mean interreinforcement time of 30 seconds. This schedule remained in effect throughout training. A criterion of 20 responses in the presence of the positive stimulus to one response (for the error group) or an attempt to respond (for the errorless group) in the presence of the negative stimulus for two consecutive days determined the discrimination was learned; that is, the total responses to positive stimulus had to be at least 20 times greater than the total responses or blackouts, depending on the group, to the negative stimulus (*3*).

Each bird was tested in extinction for generalization to the angularity negative stimulus dimension. The test stimuli were lines at angles of 30°, 60°, 90°, 120°, and 150° rotated counterclockwise from horizontal. The order of presentation of the stimuli, within each series of five stimuli, was determined randomly; nine such series were presented. Half the birds in each group were tested to the angles alone; the other ten were tested with each angle superimposed on the 555-nm light.

The lack of an inverted gradient in the responses of Terrace's errorless group may have been due to the extremely low responding maintained by his testing procedure. A test in which each test angle is superimposed on the 555-nm light can be expected to induce greater responding in the presence of those stimuli, possibly revealing differences in response strength which Terrace's procedure may have concealed.

The mean percentage of total responses for the errorless and control groups are virtually identical (Fig. 1). Since the control group had never been exposed to any angle of line, the fact that the gradient for the errorless group is similar to that for the control suggests that insofar as angularity is a "neutral" stimulus for the control group it is also a neutral stimulus for the errorless group. In agreement with Terrace's findings the gradients of the errorless and control groups show no significant differences attributable to stimuli (*4*). Only the group trained with errors showed the lowest response tendency to the 90° negative stimulus; this response depression at the negative stimulus is highly reliable (*5*).

Figure 2 presents the mean percentage of total responses for the three groups tested to each angle superimposed on the 555-nm light positive stimulus. The presence of the positive stimulus causes the gradient around the previous negative stimulus to be positive (decremental). This preference for the 90° line by both the error and errorless groups is statistically reliable (*6*). The control group again showed no preference for any angle, which indicates that angularity was a neutral stimulus dimension for this group.

Only the response rate of the error group changes as a function of the presence of the positive stimulus (*7*); the rate increases in its presence (the error group was the only one which showed a gradient of inhibition when tested to angularity alone). The fact that there are no significant changes in rate for either the errorless or the control group in the two test situations indicates again that there is no negativity (in the sense of response suppression) connected with negative stimulus for the errorless group.

Furthermore the negative stimulus is not a neutral stimulus after errorless discrimination learning; there is a positive gradient which peaks at the negative stimulus when the values from the dimension of the negative stimulus were superimposed on the positive stimulus during test. Why this should be so remains to be determined. One possibility is that during errorless training the bird became familiar with the negative stimulus training stimulus, which somehow became a positive stimulus in the context of the generalization test with positive stimulus present.

The method of bringing about errorless learning used in this study is more flexible than those used by Terrace, and it produces the same results. Discrimination training can be introduced either early or late. Subjects are not assigned to the errorless group after the fact, and elaborate programming equipment is not necessary to bring about errorless learning.

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4. Analysis of variance shows no effect of stimuli: Errorless group: $F_{4,36} = 1.37$, $P > .05$; control group: $F_{4,36} = 2.33$, $P > .05$.
5. Error group: $F_{4,36} = 4.51$. $P < .01$.
6. Errorless group: $F_{4,36} = 9.61$, $P < .01$; error group: $F_{4,36} = 8.49$, $P < .01$.
7. The t test between error group with and without positive stimulus shows $t_{(18)} = 5.35$, $P < .01$.
8. I thank D. R. Thomas for his help and encouragement. These results were presented at the annual meetings of the Midwestern Psychological Association in Chicago, 1968. This study also constitutes part of a dissertation submitted to the Graduate School at Kent State University in partial fulfillment of the requirements of the Ph.D. degree.

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Occupancy Principle: Identity with That of Mean Transit Time of Tracers in Biological Systems

Orr and Gillespie (*1*) propose application of the occupancy principle to design and analysis of experiments with tracers in steady-state biological systems. I agree with their proposal, which is especially applicable to noncompartmental systems, such as the circulation, but I am not sure that it is really different from one which I made in different language and symbols (*2*).

For those who might wish to relate the language of Orr and Gillespie to that used in certain biological fields, I wish to point out that what they call occupancy, their symbol θ, is what I and others call the mean transit time or mean residence time $\bar{t}$. In earlier tracer experiments in biology, the term "turnover time" was used widely. This term, strictly speaking, applies to systems through which the probability-density function of transit times is exponential, ke^{-kt}, in which case the turnover time is $1/k$, which is identical with $\bar{t}$.

The definition has been extended to other systems in which it is also equated with $\bar{t}$. I prefer $\bar{t}$ as less ambiguous. The dimensionless function $f(t)$, used by Orr and Gillespie, is, I

believe, the same as the function that I have been calling $[1-H(t)]$, where

$$H(t) = \int_0^t h(\tau)d\tau$$

and $h(t)$ is the frequency function, or probability density function of transit times through the distributing system, and $H(t)$ is then of course the distribution function.

Orr and Gillespie state that I applied these principles, generalized to consider any input function, to measurements of tracer concentration at the output from a system. However, I have also applied the principle, in the form

$$\bar{t} = \int_0^\infty [1-H(t)]dt$$

to measurement of tracer remaining in the system, either by the technique of external monitoring or by sampling blood within the system, and so forth. This application I called residue detection, in contrast to outflow detection. The above equation is identical with the equation for (definition of) occupancy given by Orr and Gillespie.

The proposal by Orr and Gillespie that separate "occupancy-to-capacity ratios" (that is, flows) can be obtained simultaneously by use of two or more isotopes was first made by Stephenson (*3*), first put into practice by Parrish, Hayden, Garrett, and Huff (*4*), and exploited by a number of others since. What Orr and Gillespie call capacity C is identical with either pool size, where a measure of the number of solvent particles is made, or volume, where the tracer is used to determine transit times of fluid through a system.

Orr and Gillespie state that the occupancy, or mean transit time, can be "determined even when part of it lies far beyond the time of the last measurement" by extrapolation of an apparent exponential; however this is a dangerous practice, although already used widely. There are examples in which this custom has led to gross errors, discovered when improved resolution displayed the fact that the tail of the curve deviated markedly from the expected exponential. I would avoid experiments in which major extrapolation had to be made, and urge that the time be spent better in design of experiments and methods that yield more complete information about the total curve.

In addition to the theoretical contributions by Bergner (*5*) quoted by Orr and Gillespie, as well as my own contributions along these lines, Hart proposed and used an important variant of these methods, perturbation analysis. A stimulating idea of Hart's is his formal treatment of the case in which there are partly accessible components in a multicomponent system, and in which reaction rates or flows between components are to be determined (see *6*).

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Adenosine Triphosphatase and Myopathy

The report of Brown, Chattopadhyay, and Patel (*1*) showed that erythrocyte ghosts from patients with myopathy contain a sodium plus potassium ($Na^+ + K^+$) activated adenosine triphosphatase activity which is activated instead of inhibited by ouabain at a concentration of 10^{-4} mole/liter. We have been unable to reproduce this result using our methods for the measurement of Na^+, K^+-activated adenosine triphosphatase activity in red cells of patients with muscular dystrophy of the Duchenne type.

Six normal subjects and seven patients, five of whom had an unequivocal diagnosis of Duchenne type of muscular dystrophy, were studied. Three of the patients are members of the same family. Fresh blood was collected into ethylenediaminetetraacetic acid (EDTA) (20 ml of blood per 60 mg of EDTA), the separated erythrocytes were washed, and the membranes were isolated (*2*). The adenosine triphosphatase activity of the membranes was determined in the presence and absence of $10^{-4}M$ ouabain in a final volume of 0.5 ml. There was approximately 1 mg of membrane protein per milliliter in each sample, and 2 mmole of adenosine triphosphate labeled with ^{32}P on the terminal phosphate (0.5 to 1.5 $\times$ 10^5 count min^{-1} $\mu mole^{-1}$), 2 mM $MgCl_2$, 50 mM NaCl, 10 mM KCl and 50 mM tris-HCl, pH 7.4. The differences between our method and that of Brown *et al.* are as follows. We used hypotonic lysis in distilled water (*2*) followed, in some cases, by freezing and storage for 4 days at −70°C. Brown *et al.* used

Table 1. Ouabain not an activator in red-cell membrane adenosine triphosphatase in myopathy. Patient C had a nonspecific dystrophic process and patient E, with muscle cramps, had an abnormality in forearm muscle potassium flux. All incubations were carried out in triplicate. Mean values of each determination are given. Adenosine triphosphatase is expressed as the number of micromoles of inorganic phosporus released per milligram of protein.

Experiment No.	Subjects	Status	Incubation period (min)	Adenosine triphosphatase activity		Inhibition (%)
				Control	+Ouabain	
1	P	Normal	40	0.125	0.060	51.8
2	K	Normal	40	.126	.075	40.5
3	D	Normal	40	.114	.056	51.3
4	S	Normal	40	.181	.134	26.3
5 (*6*)	S	Normal	40	.536	.481	10.1
6	R	Normal	40	.194	.087	55.4
7 (*6*)	R	Normal	40	.350	.228	33.9
8 (*7*)	H	Normal	60	.394	.314	20.2
		Dystrophy				
9	C	Dystrophy	40	0.104	0.064	38.6
10	E	Muscle cramps	40	.152	.071	53.5
11	DB	Duchenne type	40	.200	.120	39.8
12 (*6*)	DB	Duchenne type	40	.396	.340	14.1
13	PB	Duchenne type	40	.188	.098	48.0
14	KB	Duchenne type	40	.203	.104	48.4
15	N	Duchenne type	40	.142	.095	33.2
16 (*6*)	N	Duchenne type	40	.350	.252	28.1
17 (*7*)	S	Duchenne type	60	.504	.393	23.5