of A in the third position of mRNA codons has not been observed previously. Therefore, terminator codon recognition must differ in some respects from the recognition of other codons by aminoacyl-tRNA (*15*).

Further study is required to identify the kind of molecule that translates terminator-codons and the ribosomal site of translation. A protein apparently is required for termination, but its function has not been defined. It should be possible to clarify the function of R and elucidate the terminal steps of protein synthesis with the experimental approach described.

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2 August 1968

Occupancy Principle for Radioactive Tracers in Steady-State Biological Systems

Abstract. *Bergner's theoretical analysis of tracer dynamics has important applications in biology, not only for the measurement of exchangeable mass (as he demonstrated) but also for elicitation of otherwise inaccessible information on total masses of particular elements in body organs or systems—as well as in chemical engineering and geophysics.*

The occupancy principle (which we define) provides a means of determining the total flow of the material under study into a system, and the quantity of that material in any part of the system for which radioactivity can be quantitatively measured.

A steady-state system is one through which there is a continuous flow of material, and in which the quantity of material in any part of the system, and the rate of flow between any two parts, remain constant. Many biological systems behave in ways that closely approximate to this state—for example the utilization of essential dietary constituents by the human body.

Under ordinary conditions, there is no way of distinguishing one part of the entry flow into the system from another; nothing within the system indicates directly to the observer that there is in fact any flow. Such a system can be studied only when a tracer, possessing distinctive characteristics recognizable by the observer, constitutes part of the entry flow.

The occupancy principle states a relation among three parameters; occupancy, capacity, and flow. At some time t after the commencement of administration of the tracer, a defined part of the system contains a fraction $f(t)$ of the total tracer, and this fraction varies with time. The values of $f(t)$ at all times are represented simply by the curve of activity against time for the defined part of the system.

The occupancy Θ for any part of the system is defined as the total integral, with respect to time, of the tracer fraction $f(t)$ that is in that part of the system:

$$\Theta = \int_0^\infty f(t) \cdot dt$$

This is simply the total area under the activity time curve for that part of the system.

The capacity C of any part of the system is the quantity of the material under study (not the tracer) that is in that part at all times; it can be expressed in any terms appropriate to the material. Capacity has the same meaning as "amount of mother substance" or "pool size."

The material enters the system at a constant flow rate F; the entry comes, or can be regarded as coming, entirely from outside the system; therefore a recirculation cannot be part of the entry flow. The occupancy principle states that the ratio of occupancy to capacity is the same for all parts of the system and equals the reciprocal of the entry flow. The principle is valid regardless of the time course of administration of tracer; its proof is straightforward (*1*).

For extension of proof to cover the case in which the tracer is not administered instantaneously, let a fraction K of the tracer be administered at a time t after the measurement of tracer activity. The pattern of flow—that is, the shape of the activity time curve for this fraction—is independent of t. With normalization to $t = 0$, the area under the activity time curve for this fraction is also independent of t; so that the total integrated area under the normalized activity time curves, corresponding to all the fractions of tracer administered at different times t, is independent of t. Therefore the occupancy principle is valid regardless of the time course of the administration of tracer, provided that the total activity of the tracer has been measured at some time before commencement of administration, and that all activities are normalized to that time.

The problem of prolonged tracer administration has been analyzed by Bergner (*2*) for the general case, and the limited case in which only the input and output to the system are accessible has been discussed by Zierler (*3*). Use of the occupancy principle has the effect of transferring the information available from the tracer directly to the material under study. Attention is therefore focused on the real system instead

of being distracted by the ambiguities inherent in the detail of the slopes of short regions of the tracer activity curve.

Any biological system, into different points of which the tracer can be added, can be defined in different ways depending on the entry point and the entry flow. The occupancy-to-capacity ratios, of the same compartment in the different systems (defined in terms of different entry flows), can then be used for derivation of the relation between entry flows. Separate occupancy-to-capacity ratios can be obtained simultaneously by use of two or more isotopes.

In clinical studies, a small quantity of blood is commonly the most convenient part of the system for which the occupancy and the capacity can be readily obtained. Serial quantitative activity measurements of other parts of the body, with calibrated uptake counters, radioisoptope scanners, or high-resolution profile scanners, give the capacities of these other parts. If the material in the blood exists in two biochemically separable states, and if the occupancy-to-capacity ratio for the first can be measured, the occupancy for the second can be derived from the capacity, or the capacity from the occupancy.

Activity measurements cannot, of course, be carried on for infinite times. In many instances, however, an apparent final exponential decrease is reached and can be used for determination of the occupancy, provided due note is taken of implications of the assumtion that the exponential decrease continues. When the capacity is very large and the entry flow is very small, chemical estimates of the entry flow and the capacity allow the occupancy to be determined even when part of it lies far beyond the time of the last measurement. From the knowledge of the total occupancy, an effective life can be derived, or the physiological consequences of a biphasic activity curve can be deduced. Such a biphasic curve commonly results from a recirculation; in some instances in which there is significant delay before the return of tracer from a part of the system, the system can be redefined to exclude the material that is in the part. The flow of material returning must then be considered a component of the entry flow to the new system.

Biological systems deviate from steady-state behavior over short times because of irregularities of food intake and because of response to external stress. An imposed deviation occurs when the normal entry flow is so small that a useful amount of tracer, when administered instantaneously, produces major irregularity in the entry flow. These problems can be overcome by slow administration of tracer over a period of time, thus averaging out the irregularities, and allowing the system to be regarded as steady state. However, major changes (resulting from therapeutic intervention, for example) cannot be studied before elapse of sufficient time for the system to reach a new steady state. Thus, the occupancy principle, embodying a remarkably simple but far-reaching property of steady state systems, has a wide range of biomedical applications.

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1. Let a steady-state system have an entry flow F, and consider a quantity of radioactive tracer that is administered instantaneously. A fraction $f(t)$ of the tracer is contained in a defined part t seconds after the tracer enters the system. Since the fates of the tracer and the material under study are identical, the same fraction $f(t)$, of the quantity $F\ \delta t$ of the material that entered the system during a time δt, is in the defined part t seconds after entry. In other words—there will be in the system at any time a fraction $f(t)$ of the material $F\ \delta t$ that entered the system t seconds earlier. The total amount of material in the part of the system is therefore the sum of the contributions from all the material that entered the system at all times between $t = 0$ and $t = \infty$ before the defined time. Therefore

$$C = \int_0^\infty f(t)\ F dt = F \int_0^\infty f(t)\ dt = F\Theta$$

$$\Theta/C = 1/F$$

Since no particular part was defined this relation holds for all parts; it is equivalent to the one established by P.-E. E. Bergner [*J. Theoret. Biol.* **6**, 137 (1964) (equation A2.7); *ibid.* **1**, 120, 359 (1961); *Acta Radiol.* **210**, Suppl., 1 (1962); *Science* **150**, 1048 (1965)].
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Spectral Reflectance of Gull's Bill: Physiological and Evolutionary Implications for Animal Communication

Abstract. *The newly hatched laughing gull chick* (Larus atricilla) *begs food by pecking at the parent's dark red bill. The spectral reflectance of the bill over a range of 300 to 1200 nanometers reflects increasingly more with wavelength beginning about 575 nanometers. Because the chick shows a bimodal, true color preference in pecking, with modes at about 625 and 450 nanometers; the latter, blue peak in the spectral response curve is apparently not adapted to the natural stimulus of the parent's bill. The blue peak might thus be the result of limitations in the neural coding of color information in the chick's visual system.*

It is of interest in both physiological and evolutionary aspects of animal communication to discover how close a correspondence there is between the physical properties of the stimulus that serves as a social signal in nature and the differential responsiveness (stimulus preferences) of the receiver. Interactions between parent and young gulls exhibit one communication system simple enough for such analysis.

This communication system has already been partially analyzed. When ready to feed its young, the adult laughing gull (*Larus atricilla*) lowers its dark red bill in front of the chick. A hungry chick pecks at the tip of the bill, a form of begging that elicits regurgitation of partially digested food from the parent (*1*). The spectral response curve of the chick shows a maximum at about 625 nm in the orange-red and a secondary maximum at about 450 nm in the blue part of the spectrum (*1, 2*). This is a true color preference, not merely a brightness function (*1*). Furthermore, the preference curve for background is unimodal in the green, and apparently the spectral mirror-image of the curve for the stimulus-object (*3*).

Because the vegetation surrounding the gull's nest reflects green, and the parent's bill appears red to the human eye, the question arises as to why blue should also be an effective color for eliciting pecking. One possibility is that the parent's red bill actually contains a secondary blue peak of reflectance detected by the chick, even though the bill appears pure red to the human observer (as do some blue-containing red dyes).

The bills of three adult male laughing gulls—birds collected for histological studies of the reproductive organs—were removed from the specimens in the field and were immediately frozen in the dark on 21 June 1967. These were then taken to the laboratory on