total number of responses in that interval to be emitted. For example, if the response rate in some interval were constant, then the quarter-life would be simply one-fourth of the duration of the interval. That is to say, when the rate is constant, one-fourth of the responses will occur in one-fourth of the time. If the responding within an interval has positive acceleration, then the quarter-life will be greater than one-fourth of the interval. On the other hand, if the rate should show a decline within an interval, then the quarter-life will fall below one-fourth.

The quarter-life prior to injection tends to lie between 11 and 12 minutes. This means that about four-fifths of the 15-minute fixed interval has elapsed when the first one-fourth of the responses in the interval have been emitted. The high value of the quarter-life expresses the rate increase that is characteristic of fixed-interval performance.

In the first interval following injection, the average quarter-life falls increasingly further below the base-line value, for 2, 3, and 4 mg of pentobarbital, respectively. This change reflects the loss of the characteristic rate increase within the fixed interval. In the case of 3 and 4 mg of pentobarbital, the quarter-life has fallen below 3¾ minutes. This indicates that the responding in these instances shows negative, rather than the customary positive, acceleration.

The time course of the drug effect on the quarter-life is seen in the consecutive postinjection intervals. By the sixth postinjection interval, the quarter-life has almost returned to the base-line value. The effect of sodium pentobarbital, as measured by the quarter-life, is a change in the characteristic pattern of responses associated with the fixed-interval schedule. The magnitude of the change varies directly with the size of the dose and dissipates gradually in time.

The drug effect appears to be analyzable into two components: a depressive effect and a loss of the positive acceleration in responding within the 15-minute interval. The increase in responding, shown in the top part of Fig. 1, is probably a consequence of the change in the temporal pattern of responding within intervals. As the depressive effect disappears, the absence of positive acceleration produces responding that occurs throughout, rather than at the end of, the 15-minute interval, thus increasing the over-all output of responses. The fact that the depressive effect (Fig. 1, top) disappears more rapidly than the loss of discrimination (Fig. 1, bottom) probably accounts for at least some of the increase in responding.

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25 February 1957

On the Distinction between the Effects of Agents on Active and Passive Transport of Ions

Several recent studies (*1–3*) designed to distinguish between the effects of pharmacologic and other agents on the active and passive components of ionic flux have brought out the difficulties of posing this question within the framework of current definitions of active transport. A brief analysis of the assumptions on which an unambiguous distinction of this kind can be founded seems, therefore, very desirable.

The ability to separate the effects of an agent on the two components of flux hinges, of course, on an experimentally unequivocal distinction between the active or passive components themselves. However, such a satisfactory quantitative distinction does not exist at present, and one is limited to a distinction based on the thermodynamic definition of active transport as transport against an electrochemical potential gradient. This definition was given by Rosenberg (*4*). The principal objection to this formulation is that it defines active transport in terms of a *net*—that is, a necessarily unidirectional—active flux. It specifically excludes metabolically linked transport (i) that is codirectional with and in excess of that expected from the electrochemical potential gradient and (ii) that is opposing but incompletely compensating the flux resulting from the gradient. One would intuitively wish to include both of these. The definition contains nothing to justify an interpretation of the net flux as a difference between two "active" components in the two directions, as has been suggested. In addition, it encompasses transport processes that require a supply of energy but do not derive it from metabolic reactions—for example, those leading to a Donnan equilibrium.

It may seem that one could define active transport as transport specifically coupled with metabolic reactions, without reference to the direction of the resulting net flux. However, in the absence of a precise knowledge of the mechanisms involved or of a possibility of measuring "passive" permeability coefficients in the *undisturbed* living system, such a definition does not prove experimentally meaningful. Numerous attempts have been made to arrive at a value for the thus defined "passive" term through the use of metabolic inhibitors. That it is, however, impossible in principle to make an unequivocal distinction between "active" and "passive" or even "metabolically dependent" and "metabolically independent" components of flux *solely* on the basis of experiments with metabolic inhibitors can be seen from the following considerations.

According to the most general definition, the isotopically measured flux in either direction would be expressed as a sum of a "passive" and an "active" term. The passive term should in principle be given by the product of a permeability coefficient, which is determined by the properties of the cell membrane and the activity of the ion on the side from which the flux originates. In the absence of evidence to the contrary —and such evidence is unobtainable without a clear-cut identification of the "active" term—the permeability coefficient must be assumed to depend on the state of metabolism as well as on ionic activity. Any metabolic inhibition must therefore be assumed to alter the permeability coefficient by an unknown amount and in an unknown direction. Thus one may not regard its measured value even as a meaningful extremum, making a distinction between the "active" and "passive" terms on this basis impossible.

More difficult to foresee is the impossibility of distinguishing by this approach between the components of flux which *are* and those which *are not* dependent on metabolism. We may represent the total (measured) flux by an equation such as

$$f_i = p^o\pi(m)\,a_o + f_i^*(m) \qquad (1)$$

where f_i is the total inward flux, p^o the value of the permeability coefficient in the complete absence of metabolism but with all other independent variables returning their values, $\pi\ (m)$ an unknown function describing its dependence on the rates of metabolic reactions, a_o the activity of the ion on the outside, and $f_i^*(m)$ the unknown active transport term. Even if perfect metabolic inhibition were achieved, there is no means of ruling out any effect of the inhibitor on the membranes, which itself is independent of metabolism, so that again the measured value $p^{o\prime}$ may in no way reflect even an extreme value of p^o. In other

Table 1. Survey of possibilities. If $R_f' = R_f$, there is proportional enhancement of both active and passive transport of proportional depression of both, or no effect.

$R_f' > R_f$		$R_f' < R_f$	
Condition	Result	Condition	Result
A. f_i increased, f_o unchanged	Enchancement of active transport	*A.* f_i decreased, f_o unchanged	Depression of active transport
B. f_o decreased, f_i unchanged	Depression of passive term, exactly compensated by enhancement of active term	*B.* f_o increased, f_i unchanged	Enhancement of passive term, exactly compensated by depression of active term
C. f_i increased more than f_o	Enhancement of both active and passive transport	*C.* f_i increased less than f_o	Enhancement of passive transport
D. f_i decreased less than f_o	Depression of passive transport	*D.* f_i decreased more than f_o	Depression of both active and passive transport
E. f_i increased, f_o decreased	Enhancement of active transport and depression of passive transport	*E.* f_i decreased, f_o increased	Depression of active term and enhancement of passive term

words, all parameters in Eq. 1 have to be considered as functions of the newly introduced variable, the agent, even if they would represent meaningful mutually exclusive terms in its absence. Consequently any definition that requires the introduction of an additional variable breaks down on a priori grounds. Thus a distinction between the effects of an agent on the two separate components of flux, if it is to accomplish its purpose, should be based on an independent, physical definition of active transport, such as that of Rosenberg. This can be accomplished under the following conditions.

For reference, the defining equation for the active-transport term can, in analogy to the equation derived by Ussing (*5*) be written in the form

$$R_f = \frac{f_i}{f_o} = \frac{P_i a_o + f_i^*}{P_o a_i} \qquad (2)$$

where f_i is the isotopic inward flux, f_o the outward flux, f_i^* the "active" flux, a_o and a_i the electrochemical activities outside and inside, respectively, and P_i and P_o the inward and outward permeability coefficients. Equation 2, really a composite of two equations, contains one more undetermined variable than the number of equations. Hence, if one is to decide whether active transport occurs in a given case, a different expression, containing only measurable quantities, has to be used. Since the only restriction on the relationship between fluxes and activities imposed by the definition of active transport is that $f_i > f_o$ as long as $a_o > a_i$ and vice versa, given a permeable membrane, such an expression is given by

$$\frac{R_f - 1}{R_a - 1} > 0 \qquad (3)$$

where $R_a = a_o/a_i$. As long as this relationship is satisfied, transport is to be regarded as passive. Equation 3 is less restrictive than that derived by Ussing (*5*), but it is the only obligatory statement of the afore-mentioned definition of active transport. Glynn (*6*) has made use of two equivalent inequalities in discussing possible deviations from Ussing's equation, without, however, pointing out that they could be properly used in defining active transport. Implicitly, the relationship of Eq. 3 has been used by Hodgkin and Keynes (*3*) in deciding that the transport of K^+ in the giant squid axon was passive after administration of dinitrophenol. The latter authors have also pointed out that Ussing's original equation requires further assumptions and does not furnish a criterion for the occurrence of active transport, except in special cases. It might be noted that the only condition under which Ussing's equation is valid by thermodynamic arguments alone is $a_o = a_i$, for which Eq. 3 becomes indeterminable. This is also the only condition under which the active transport term can be numerically evaluated, since it does not follow from thermodynamic arguments that the permeability coefficients in the two directions (both being functions of concentration) are equal, except at $a_o = a_i$.

Once the occurrence of active transport in a given system has been ascertained, an attempt to separate the effects of an agent on the active and passive components is possible only on the further assumptions that the agent affects the inward and outward permeability coefficients in the same sense and in equal proportions and does nothing to alter the activities. Experimentally, this requires the measurement of the flux ratio R_f in the absence and in the presence of the agent. If the agent depresses the permeability coefficients, without an effect on active transport f_o' will be decreased more than f_i', so that $R_f' > R_f$, the quantities measured in the presence of the agent being designated by primes. A decrease of active transport alone will depress f_i', but leave f_o unaltered, so that $R_f' < R_f$. Depression of both the active and passive term will also yield $R_f' < R_f$, but accompanied by a decrease in both f_i' and f_o'. A complete survey of possibilities is tabulated in Table 1. Except in the case where no change of the flux ratio is observed, the four measurements combined with the demonstration of constant activities provide a rather unambiguous separation of the effect of an agent on the active and passive components of the total flux, as defined thermodynamically. It must be kept in mind, however, that this definition and the procedures based on it in no way reflect effects on all the metabolically linked components which may actually occur in living systems. They are of value only insofar as the latter type of information is not obtainable at present with any degree of reliability (*7*).

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7. I am very much indebted to A. M. Shanes and C. A. M. Hogben for a critical discussion of the manuscript. This article is contribution No. 2144 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology.
* National Research Council fellow.

18 February 1957

Diathelic Immunization—a Maternal-Offspring Relationship Involving Milk Antibodies

The extraordinary speed with which the mammary gland returns specific antibody upon direct immunization with bacterial antigens has led us to experiments testing the hypothesis that in intercurrent infections of the young, injection of the antigen through the teat orifice during the act of nursing may lead to the return of specifically immune milk. Nine normally lactating cows were used, and in each experiment a live culture of *Salmonella pullorum* was instilled into the mouth of a calf during a single nursing. This species of bacteria was chosen because it is not a resident or pathogen of cows. Only two quarters of the udder were offered to the calves in each instance. The organisms were prepared as washings from 24-hour cultures on veal broth slants. Testing of the milk