ON THE CALCULATION OF "TURNOVER TIME" AND "TURNOVER RATE" FROM EXPERIMENTS INVOLVING THE USE OF LABELING AGENTS

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Although labeling agents have been widely used to characterize the course of reactions in the animal body, their application to quantitative aspects of the turnover of a substance has been slow to develop.

If a certain fraction of the administered isotopic substance[1] is incorporated into a compound, *at least* the same fraction of the total administered labeled substance[2] must have been converted into that compound. Such a calculation gives a minimum value for the conversion of a labeled substance into a compound. Fishler (1) showed that the fraction of the administered P^{32} recovered in the phospholipid of liver, muscle, or blood of rats after a 12 hour interval was the same even though the amounts of labeled phosphate injected varied from 6 to 48 mg. This demonstrates that the administered labeled phosphate was negligible in comparison with the phosphate available for incorporation into phospholipids in the animal body. It must therefore be obvious that such minimum values have little significance, for when the amount of injected labeled substance was *varied eightfold* the minimum value was altered to the same extent.

For the above type of calculation to yield a correct measure of the amount of labeled atoms[2] incorporated into a substance, the amount of labeled molecules injected should be large enough to render negligible the amount of those molecules already present in the organism. Such a procedure, however—namely one in which the amount of injected substance is large enough to yield a correct measure of the amount of newly formed compound—would probably disturb the normal metabolism of the organism.

Artom *et al.* made an interesting contribution to this field, well realizing the difficulties involved in simplifying the complex system in which most of the biological reactions occur (2).

By means of repeated injections of P^{32} Hevesy and Hahn (3) maintained a constant specific activity of inorganic phosphate in the plasma. They assumed

[1] Isotopic molecules (−substance) = all the molecules (substance) containing the particular isotopic atom.

[2] Labeled atoms (−molecules, −substance) = all the atoms (molecules, substance) mixed with, and chemically indistinguishable from, the isotopic atoms (−molecules, −substance).

that the same constant specific activity of the immediate precursor was maintained at the site of the reaction. Whether this is the case is not known. From the ratios of the specific activity of the organ phospholipid to that of plasma phosphate, they obtained the fraction of newly formed phospholipid molecules formed in that organ. They failed to take into account the breakdown of newly formed molecules; hence their calculation is applicable only to experiments of very short duration with respect to the turnover time of a given substance.

In the present communication, a simple method for the determination of the turnover rate of a substance and the identification of its precursor is presented.

Terminology and Assumptions

The following terms are used in the present treatment:

Specific Activity.—The specific activity (s.a.) of a substance containing a labeled atom, L, is the amount of radioactive L (radioactive units) per unit of labeled L (mg.) present.

Turnover.—This term refers to the process of renewal of a given substance, which may be accomplished in the following ways: (1) The incorporation of labeled atoms or radicals into a substance; *i.e.*, synthesis or exchange. (2) The entering of a labeled substance into a tissue; *i.e.*, transport. (3) A combination of the above two processes, which may be termed here appearance of a substance.

Turnover Rate.—The turnover rate of a substance in a tissue is the amount of the substance that is turned over by that tissue per unit of time.

Turnover Time.—The turnover time of a substance in a tissue is the time required for the appearance or disappearance of an amount of that substance equal to the amount of that substance present in the tissue. If, for example, the rate of appearance of a substance in a tissue is "a" and the amount of that substance present in that tissue is "b," the turnover time will be "$\frac{b}{a}$."

The following assumptions are made in the calculations below:

(*1*) *Steady State.*—The amount of compound present in the tissue studied must be constant during the interval over which the calculation is made; *i.e.*, the rate of appearance of the compound must *equal* its rate of disappearance.

(*2*) *Constant Rate of Appearance and Disappearance.*—The rate of appearance and disappearance of the compound must be constant during the time interval used for the calculation.

(*3*) *Random Appearance and Disappearance.*—The appearance and disappearance of all molecules must proceed at random; *i.e.*, the organism does not distinguish between "old" and "newly" formed molecules. In the case of phospholipids Hevesy and Hahn (3) appear to assume that such a distinction is made in the animal organism, but this seems very unlikely, especially in tissues where little or no organization exists such as plasma. The assumption made in the present study implies that the specific activity of the compound formed (or

entering) at any time is equal to the specific activity of its immediate precursor[3] at that time, and that the specific activity of portions of the compound breaking down (or leaving the tissue) is equal to the specific activity of the total amount of the compound present in that tissue.

A. Criteria for the Establishment of a Precursor

It is known that in a biological system one deals with dynamic equilibrium mixtures of all types of molecules at different energy levels. Isolation of a com-

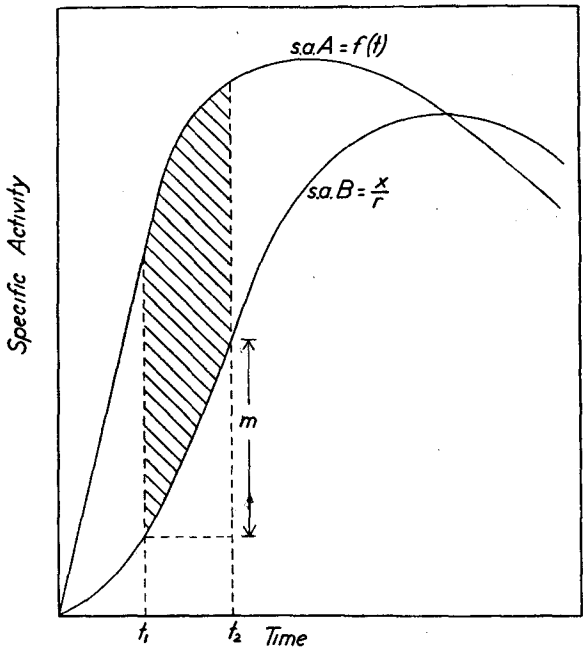

FIG. 1. Illustration of the "s.a.-time" relations of precursor A and product B.

pound from such mixtures undoubtedly involves the shifting of these equilibria towards that more stable compound. If, therefore, the immediate precursor A of a compound B can be isolated by chemical procedures, it means that A is the *last "stable" compound* which precedes the formation of B. Compound B may have more than one immediate precursor, because two or more molecules may combine to form B. At present, however, we are concerned only with the precursor A containing the labeled atom, and we therefore will call it *the* immediate precursor of B.

It has been pointed out frequently that during the early interval after the administration of a labeled substance the s.a. (or isotopic concentration, if nonradioactive isotopes are employed) of a precursor of a compound must be

[3] The term immediate precursor is explained below.

higher than that of the compound itself. It has been noted too that, if the s.a. of the precursor is maintained constant, the s.a. of the compound eventually becomes equal to that of the precursor.

It will now be shown how an immediate precursor can be determined more precisely from the "s.a.-time" relations of the precursor and compound. Let us again consider the case in which a single immediate precursor A is converted to compound B.

Let

p = the rate of conversion of A to B (assumed to be constant).
r = the amount of B present in the tissue (assumed to be constant).
x = the amount of radioactive B present in that tissue.
$f(t)$ = the s.a. of the immediate precursor A, which, as expressed here, depends on time.

Then the amount of radioactivity that will be converted into B per unit of time is $pf(t)$, and the amount of radioactivity that is lost from B per unit of time is $p\frac{x}{r}$. Therefore, the rate of change of the amount of radioactivity in B in a tissue per unit of time =

$$\frac{dx}{dt} = pf(t) - p\frac{x}{r} = p\left[f(t) - \frac{x}{r}\right] \quad \text{or}$$

$$r\left(\frac{d\frac{x}{r}}{dt}\right) = p\left[f(t) - \frac{x}{r}\right] \quad \text{since } r \text{ is constant}$$

$$\text{and} \quad \frac{\left(\frac{d\frac{x}{r}}{dt}\right)}{f(t) - \frac{x}{r}} = \frac{p}{r} = \text{a constant.}$$

$\left(\frac{d\frac{x}{r}}{dt}\right)$ measures the slope of the "s.a.-time" curve of B. We may now deduce the following relation between the s.a. of the compound B and the s.a. of its precursor A: *At any time the slope of the "s.a.-time" curve of B is proportional to the difference between the s.a. of A; i.e., $f(t)$, and the s.a. of B, i.e. $\frac{x}{r}$.*

The application of this relation in the case in which a single dose of labeling agent is administered is illustrated in Fig. 1. In general the following three criteria for an immediate precursor will be most useful.

(1) If the slope of the "s.a.-time" curve of B is positive (see Fig. 1), *i.e. before* the s.a. of B reaches its maximum, $\left[f(t) - \frac{x}{r}\right]$ must be positive. This means *that the s.a. of the immediate precursor A is greater than that of the compound B before the latter reaches its maximum s.a.*

(2) *After B has reached its maximum s.a.*, the slope of the "s.a.-time" curve of B is negative, and therefore *the s.a. of the compound is greater than that* of its precursor.

(3) At the time *when B has reached its maximum s.a.* the slope of the "s.a.-time" curve of B is zero and therefore *the s.a. of the immediate precursor A equals the s.a. of compound B* at that time.

B. Calculations of Turnover Time

The general equation derived above was

$$\frac{r}{p}\left(\frac{d\frac{x}{r}}{dt}\right) = \left[f(t) - \frac{x}{r}\right] \tag{1}$$

As defined above, turnover time (which will be designated by t_t) $= \frac{r}{p}$. If the "s.a.-time" curves of A and B are known, it is possible to determine $\frac{r}{p}$ from equation (1).

Since the determination of the slope $\frac{d\frac{x}{r}}{dt}$ involves an error much larger than the experimental error in $\frac{x}{r}$, it is advisable to use the integrated rather than the differential equation:

$$\frac{r}{p}\int_{t_1}^{t_2} d\frac{x}{r} = \int_{t_1}^{t_2} f(t)dt - \int_{t_1}^{t_2} \frac{x}{r}dt$$

$$t_t\left(\frac{x_2}{r} - \frac{x_1}{r}\right) = \int_{t_1}^{t_2} f(t)dt - \int_{t_1}^{t_2} \frac{x}{r}dt = \text{shaded area}$$

$$t_t = \frac{\text{shaded area}}{m} \qquad \text{(see Fig. 1.)}$$

From Fig. 1, it can be seen how t_t can be determined. There are cases, however, in which it is advisable to use the ratios of the s.a. of B to the s.a. of A rather than their absolute values. This will be the case, for example, when data from more than one animal are used in the calculation, since the above

ratios tend to be more uniform from animal to animal than the s.a. themselves. An illustration of the use of these s.a. ratios will be given in the following two cases.

The analytical solution of equation (1) is

$$xe^{\frac{p}{r}t} = \int pf(t)e^{\frac{p}{r}t}\,dt + C.$$

For the case where the s.a. of the immediate precursor is maintained constant (*i.e.* $f(t) = a$), this becomes $\frac{x}{r} = a\left(1 - e^{-\frac{p}{r}t}\right)$, since $x = 0$ when $t = 0$. At turnover time $t = \frac{r}{p}$ and therefore $\frac{x}{r} = a(1 - e^{-1}) = 0.63\,a$, *i.e.* the ratio of the $\frac{\text{s.a.}B}{\text{s.a.}A}$ $= 0.63$ at turnover time; and similarly at $\frac{1}{n}$ of the turnover time

$$\left(i.e.\ \text{where } t = \frac{t_t}{n}\right)\frac{\text{s.a.}B}{\text{s.a.}A} = \left(1 - e^{-\frac{1}{n}}\right) \tag{2}$$

When the s.a. of the immediate precursor varies linearly with time (*i.e.* $f(t) = bt$), the solution is simplified to

$$\frac{x}{r} = \frac{br}{p}\left(\frac{p}{r}t - 1 + e^{-\frac{p}{r}t}\right) \text{ since } x = 0 \text{ when } t = 0.$$

At turnover time again $t = \frac{r}{p}$ and $\frac{\text{s.a.}B}{\text{s.a.}A} = \frac{x/r}{bt_t} = 0.37$,

and at $\frac{1}{n}$ turnover time $\frac{\text{s.a.}B}{\text{s.a.}A} = \frac{x/r}{bt_{t/n}} = n\left(\frac{1}{n} - 1 + e^{-\frac{1}{n}}\right)$ (3)

From a given ratio of the s.a. of B to the s.a. of A at the time interval T we can determine "n" from equation (2) or (3), and from $T = \frac{t_t}{n}$ the turnover time of the given substance B can be determined.

If the total amount of substance B present in that tissue is known ($= r$), the turnover rate "p" can be obtained from the equation $\frac{r}{t_t} = p$.

The Rate of Disappearance of a Compound as a Measure of Turnover Rate.—If the turnover rate of a substance B in the circulating fluid has to be determined, it is convenient to measure its rate of disappearance from the circulating fluid. From the "steady state" assumption we know that the rate of appearance of B in the circulating fluid must equal its rate of disappearance, so that the latter gives a true measure of the turnover rate. The advantage of this method is that the immediate precursor of the compound B does not have to be known.

The measurement can be performed by the introduction of a small amount of

labeled substance B into the circulating fluid and by determining its s.a. at different time intervals thereafter.

Let

p = rate of disappearance of B from the circulating fluid,
x = the amount of radioactive B (in r.u.) present in the circulating fluid at any time,
r = the total amount of B present in the circulating fluid (assumed to be constant);

then

$$\frac{dx}{dt} = -p\frac{x}{r} \text{ and on integration } \frac{x}{r} = ce^{-\frac{p}{r}t};$$

taking the natural logarithm on both sides, $\ln \frac{x}{r} = \ln c - \frac{p}{r}t$. It is clear that a plot of $\ln \frac{x}{r}$ ($=\ln$ s.a.B) against t will yield a straight line whose slope will be $= -\frac{p}{r} = -\frac{1}{t_t}$. (4)

From equation (4) the turnover rate p can also be determined if the total amount of B present in the circulating fluid ($= r$) is known. The latter quantity can be calculated from the data obtained in this type of experiment, as will be shown in the following paper dealing with the determination of the turnover of phospholipids in the plasma of dogs. It should be kept in mind that the above relations will hold only during a time interval in which no appreciable amount of isotopic substance returns from the tissues to the circulating fluid.

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SUMMARY

1. A new method for the determination of an immediate precursor of a substance occurring in the animal body is presented.

2. Calculations on the quantitative determination of the rate of turnover of a substance and their application to experiments involving the use of labeling agents are given. These calculations take into account loss of the isotopic substance by way of breakdown or transport.

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