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

hypotonic lysis in EDTA. In addition, in experiments 8 and 17, we also followed the method of membrane preparation and incubation of Brown *et al.*, except that we used 3 mg of EDTA per milliliter compared to 0.6 to 1.2 mg/ml. Incubation was carried out for 40 to 60 minutes at 37°C with air as the gas phase, compared to 20 minutes at 42°C used by Brown *et al.* The reaction was stopped by the addition of 0.5 ml of 10 percent trichloroacetic acid. After centrifugation, the amount of inorganic ^{32}P liberated in the protein-free supernatant was determined by the radioactivity method of Siegal and Albers (*3*). We also used this method for the determination of the release of inorganic orthophosphate in the two experiments 8 and 17 in which we reproduced Brown's method; Brown *et al.* used a chemical method. All incubations were carried out in triplicate. Mean values of the adenosine triphosphatase activity in control experiments as well as in experiments with ouabain added are shown in Table 1. In each case, the Mg^{++}-dependent, Na^+, K^+-activated adenosine triphosphatase activity was inhibited in the presence of ouabain.

Freezing and thawing of the erythrocyte ghosts (experiments 5, 7, 12, and 16) increased the enzymic activity, but ouabain inhibition was still observed. In this last-named group of studies, the lesser degree of inhibition is consistent with the further release of only Mg^{++}-dependent adenosine triphosphatase activity if the membranes are disrupted further (freezing). When we followed Brown *et al.*'s method of membrane preparation and incubation (experiments 8 and 17), we did not observe either the high adenosine triphosphatase activity reported (*1*) or the absence of ouabain inhibition of activity. No significant differences were detected in either the degree of enzyme activity or in the ouabain inhibition when the cells of patients were compared to those of normal control subjects.

The high activities of adenosine triphosphatase reported by Brown *et al.* (*1*) (200 nmole of inorganic phosphorus liberated per minute per milligram of protein) are difficult to explain; that is, stoichiometrically, there was more inorganic phosphate released than there was sodium adenosine triphosphate added, calculated on the basis of the amount of membrane protein in their medium and the total incubation time of 20 minutes.

Stimulation of Na^+, K^+-activated adenosine triphosphatase activity has been reported (*4*), but only with the ouabain concentrations of approximately 10^{-10} mole/liter. Although altered (decreased) adenosine triphosphatase activity in erythrocyte membranes in uremic patients with hemolytic anemia has been reported (*5*), this condition has not been documented in patients with muscular dystrophy. It has not been possible to correlate the data of Brown *et al.* (*1*) with either the above observations (*4, 5*) or with our data.

Our experience suggests that the Na^+, K^+-activated adenosine triphosphatase activity of erythrocyte ghosts from normal subjects and from patients with muscular dystrophy are inhibited by ouabain in a concentration of 10^{-4} mole/liter.

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The specifics of our method were chosen to optimize the ouabain-response phenomenon. Klassen and Blostein did not follow the procedure we described. They used different metal concentrations, different buffer concentration, different incubation time, and temperature, and they omitted sucrose from medium.

Our legend (Table 1, p. 1578) should read 10^{-8} mole. Conditions chosen, while different from some used by other workers, are nonetheless well within the bounds of enzymology convention.

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Aquaculture: Amplification and Correction

Inquiries from colleagues received since my article on aquaculture appeared (*1*) suggest the following corrections and amplifications: (i) to page 1100, column 3, paragraph 2; and (ii) to page 1102, column 3, paragraph 3, and to Table 1.

With regard to (i), the enormous numbers of food organisms mentioned as necessary to sustain tank-reared pelagic fish larvae were based on the assumption that every cubic centimeter in the entire water volume must contain sufficient numbers of plankters at all times even though only a limited quantity of them is removed by grazing. The Fishery-Oceanography Center of the Bureau of Commercial Fisheries at La Jolla where the experiments I mentioned were undertaken informs me that it is not necessary to make such massive collections of plankton for rearing the relatively small numbers of larvae which they require for the purpose of gaining insight into problems of larval survival at sea. The culture of pelagic fish larvae by the bureau was and is not conducted with aquaculture in mind but to shed light on mechanisms which control year-class strength of pelagic fish stocks.

With regard to (ii), the per hectare production of oyster flesh in both the above mentioned places is at variance with other, supporting figures in my text that would indicate it to be 20 and not 58 metric tons. I regret to have made a mistake in the conversion of figures from British and Japanese measuring systems into the metric one. On checking again with the Hiroshima Provincial Fisheries Laboratory, I find that the per hectare yields of oyster flesh from rearing sites in the Japan Inland Sea in fact range from 12 to 30 metric tons with an average of 18 to 20 metric tons: the per hectare dollar value of these oysters would then be $23,100 and not $67,000, as stated in the table. It should be noted once more that this harvest comes from an open aquatic ecosystem with tidal interchange, and the primary production under a much larger area of water than that of the rearing sites proper is responsible for it.

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