LETTERS TO THE EDITOR

Reply to "Comments on Electrical Fluctuations Associated with Active Transport"

Dear Sir:

Fishman and Dorset (1973) raise several questions concerning the methodology of my paper (Segal, 1972) which I believe are answered as follows.

It is quite possible, as they suggest, that a more optimal method might be developed for the detection of the electrical fluctuations associated with active transport across frog skin. As regards my paper, the issue is whether or not my experimental arrangement obfuscated the noise of the skin sample. Fishman and Dorset concede that my amplifier noise power was two or three orders of magnitude less than that which I attributed to the skin (note that their statement of my units of spectral intensity is incorrect). I continuously assessed and accounted for the total noise of my system (electrodes, amplifiers, filter, etc.) as described in par. 3, p. 1374; par. 4, p. 1384; and par. 2, p. 1385. In addition, not mentioned in my paper, is the fact that the electrical fluctuations I found always disappeared as the sample died. That is, after a day or so the noise diminished to the amount observed when no sample was present. Furthermore, ouabain eliminated the voltage fluctuations of the sample (par. 4, p. 1379; par. 2, p. 1380). It is difficult, therefore, to attribute the noise I found to the electrodes and/or the ensuing electronic components.

Their assertion that the low frequency flattening of the upper graph of Fig. 2 arises from the limited frequency response of the amplifier takes no cognizance of the calibration procedure I employed (par 4, p. 1373). The method compensates for the frequency response of the amplifiers and filter, as well as the frequency dependence of the bandwidth of the latter.

In any case, restricted amplifier bandwidth cannot explain the fact that only the upper graph of Fig. 2 shows the flattening. The graph of Fig. 1 and the lower one of Fig. 2 do not have this feature. Yet the sole difference between the measuring conditions of the three curves is that for the upper graph of Fig. 2 the temperature of the skin sample was raised from 20°C to 32°C, as stated in the figure legends.

The formula Fishman and Dorset use to support their claim that my period of measurement for a single determination of spectral intensity was too brief is, at best, only approximately applicable to my system. It assumes integration by a single RC circuit of ΔV^2 and a filter with a square passband (Ziel, 1954), whereas I directly integrated the absolute value of ΔV, and the passband of my filter more closely resembled that of a sharply tuned resonance circuit. As van der Ziel has shown, theoretical accuracy varies with such parameters. Rather than deriving an expression for the theoretical accuracy of a single measurement, and in view of the generally large variability between biological samples, it is more germane to ask whether or not my actual data, taken as a whole, are sufficiently accurate to allow the inferences I drew therefrom.

The data points to my Fig. 1 are not individual measurements but the means of 19 determinations on 12 individual skin samples; those of the upper graph of Fig. 2 are the means of six determinations on five samples. In both cases the standard errors of the means are indicated. From Fig. 1 I concluded that the points fell along a straight line; from Fig. 2 that

the spectrum became sigmoid shaped and that the slope at the inflection point increased with respect to the slope of the graph of Fig. 1. It is evident from my Figs. 1 and 2 that the SEM's of the data points are too small to obviate these conclusions.

It should be pointed out also that the integration period for the individual measurements comprising the data points of Fig. 2 was, by virtue of the time compression method, 40 min, not the 5 min discussed by Fishman and Dorset. Fig. 4 which does contain the results of single measurements, rather than the means of many, does not display anything like the degree of scatter Fishman and Dorset envision. Fortunately, too, since fluctuation spectra are plotted logarithmically the graphic effect of data variability diminishes greatly.

I employed the porous glass disk solely as a temperature transducer—not, as Fishman and Dorset state, for a "check of [my] measurement system. . . ." My somewhat gratuitous conclusion that its noise was "about equal to the theoretical Johnson noise . . ." was certainly not intended as a definitive generalization covering the electrical fluctuations of electrolyte systems. I regret any inconvenience or misconception caused by that statement.

Ouabain does have an effect on the fluctuations of frog skin. The data of par. 4, p. 1379 and par. 2, p. 1380 show that the fluctuations attributed to the current flow arising during active transport vanish when the skin is treated with ouabain, but that those of the skin's electrical resistance do not. The possible relationship of both of these findings to the mechanism of active transport, particularly carrier-mediated transport, is treated in detail in par. 3, p. 1386, *et seq.*

Reasons for concluding that the fluctuations I observed reveal the mechanism of active transport are discussed in full throughout my paper. That some inorganic membranes, under appropriate conditions, generate noise which is similar to that of frog skin does not, per se, vitiate my conclusions. There is nothing implicit in the concept of active transport which requires that all details of its mechanism be unique to a metabolizing biological system.

Received for publication 21 August 1973 and in revised form 25 January 1974.

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Fluid Flow through Small Pores

Dear Sir:

The sole argument presented by Schindler and Iberall (1973, *Biophys. J.* 13:804) against the validity of continuum hydrodynamic fluid flow in channels of *ca.* 80 Å diameter is misleading. According to Eq. 3 in their paper, which itself is a reasonable estimate of the mean square displacement of a molecule by random diffusion movements in a continuous medium (derived from classical kinetic theory), they calculate that in the time required for a water molecule to flow through a capillary pore 80 Å in diameter and 0.1 μm long (5 ms), its displacement due to diffusion would greatly exceed the pore diameter. They conclude that "in-