LETTERS TO THE EDITOR

Comments on "Electrical Fluctuations Associated with Active Transport"

Dear Sir:

In a recent communication (Segal, 1972) measurements of fluctuations in the potential differences across isolated abdominal frog skin were reported. It was suggested that these fluctuations are associated with active transport in the epithelium. Based upon our experience with measurements of fluctuation phenomena in some biological (Fishman, 1972, 1973 *a*, *b*) and synthetic (Dorset and Fishman, 1972) membranes, we wish to comment on some of the measurement conditions and sources of error which make the results presented in this paper uncertain. Our criticism is summarized in three points: (*a*) the experimental arrangement was not optimal for noise measurements and amplifier noise was underrated; (*b*) the low frequency noise-power measurements were inaccurate; and (*c*) many synthetic membranes produce similar low frequency noise spectra.

First, we consider some of the measurement conditions and sources of error. The potential difference across frog skin was "detected via Ag-AgCl electrodes immersed in Ringer's solution and connected to the fluid in the half-cells by 4% agar-Ringer's solution bridges." Since frog Ringer's is not a highly conducting solution ($\rho = 80\ \Omega$ cm), the total access resistance from electrodes to the skin surfaces was probably equal to or greater than the measured skin resistance (1–5 $k\Omega$). These access resistances produce thermal noise and perhaps additional electrode noise. Such conditions are not optimal (Poussart, 1973) for observing noise from the skin alone. A preferred method would be to isolate very small areas of frog skin (to increase the magnitude of fluctuations) without increasing the access resistances to the isolated area (Fishman, 1973 *b*).

With respect to instrumentation, we note that it is not possible to observe the thermal noise of low resistances (kiloohms) with the specific preamplifier (Princeton Applied Research Corp., Princeton, N.J., model 113) used. Fig. 1 shows measured power density spectra $S(f) = \overline{\Delta V^2}/\Delta f$ vs. f, where $\overline{\Delta V^2}$ is the mean square value of the spectral component at frequency f measured with instrumental bandwidth Δf for the PAR model 113. These spectra were obtained with a real-time spectrum analyzer (Signal Analysis Industries Corp., Hauppague, N.Y., model 52) which uses a time compression system to obtain subaudio and audio spectra rapidly. Furthermore, this analyzer provides a 400 point spectrum in each analysis band with excellent resolution (e.g. 0.05 Hz in the frequency band 0.1–20 Hz) as well as spectral averaging to increase the statistical accuracy. From Fig. 1, it is apparent that the noise from the short-circuited PAR 113 alone in the frequency range 0.1–10 Hz is greater (10^{-15}–10^{-14} V^2/Hz) than the thermal level predicted by the Nyquist (1928) relation for skin resistances of 1–5 $k\Omega$ (power densities of the order of 10^{-17} V^2/Hz). Consequently, it is unlikely that the thermal noise or noise during current flow through substitute membranes (with resistances equivalent to the skin resistances) could have been determined accurately with a PAR 113. Nevertheless, the observed noise may still be associated with the skin since the noise level was 1.54×10^{-13} V^2/Hz at 1 Hz (Fig. 1, Segal, 1972).

A more important point is that the low cutoff frequency (-3 dB point) of a standard AC-coupled PAR 113 is 0.03 Hz and not 0.01 Hz as stated in the Methods section. Consequently, the PAR 113 cutoff frequency was within the pass-band (which was extended to 0.025 Hz by a time compression method) of the measured spectral components. This condition would

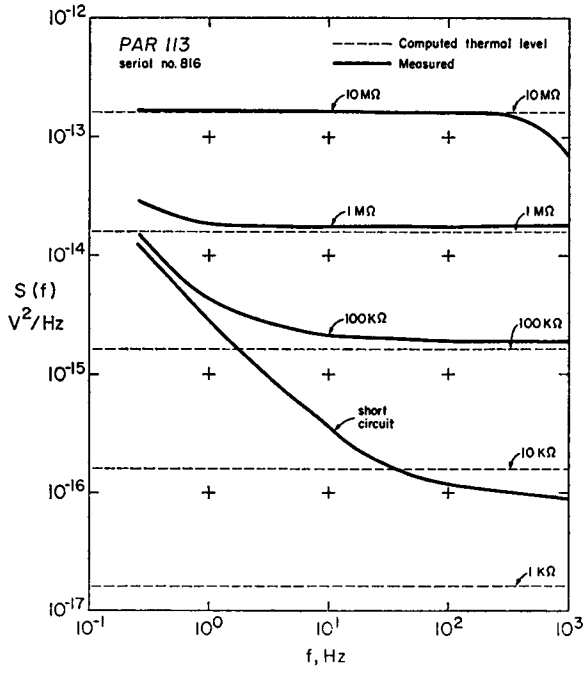

FIGURE 1 Power density spectra of the PAR 113 preamplifier (single-ended) with a pass-band of 0.03 Hz to 300 kHz and the input terminated with the indicated resistances. Thermal levels calculated from the Nyquist relation at 20°C.

produce measured spectral intensity values which would be significantly lower than the actual values and could create the illusion of a low frequency spectral corner—frequency such as in the spectrum at 31.9°C (Fig. 2, Segal, 1972).

Secondly, the use of a constant percentage-bandwidth filter for spectral analysis at sub-audio frequencies can produce large errors (Sutcliffe, 1965). In order to obtain a reasonably accurate (±10%) mean square value of the frequency components passed by a narrow filter (centered at 1 Hz or less) long observation times are required. Sutcliffe shows from theoretical considerations that the standard deviation for such a measurement is approximately equal to $(Bt)^{-1}$, where B is the filter bandwidth and t is the observation time. Thus for a 10% SD of the true noise power at a center frequency of 0.1 Hz and a bandwidth of 0.012 Hz (Segal states that the minimum filter bandwidth was ±6% of center frequency), the required observation time is 8,333 s (2.3 h), whereas the actual observation time at this frequency was only 300 s (5 min). Similarly, the observation times at other frequencies throughout the measurement frequency band (0.1–10 Hz) were not long enough to obtain an accurate mean square value. Without accurate determination of the mean square amplitude of the components in the fluctuating potential, the shape and slope of the resulting spectra as well as the subsequent interpretations based upon these spectra are in question.

Finally, as a check of his measurement system, Segal used a porous glass disk (holes with diameter of 10 μm) from which he observed only thermal noise for solutions which gave resistances comparable with the skins used. Despite the spectra in Fig. 1, which indicate that 1–5 kΩ thermal levels could not be measured with the PAR 113, the measurement of Hooge and Gaal (1971) and DeFelice and Michalides (1972) as well as our measurements, which are summarized in Fig. 2 (Dorset and Fishman, 1972), on noise in electrolytic solutions

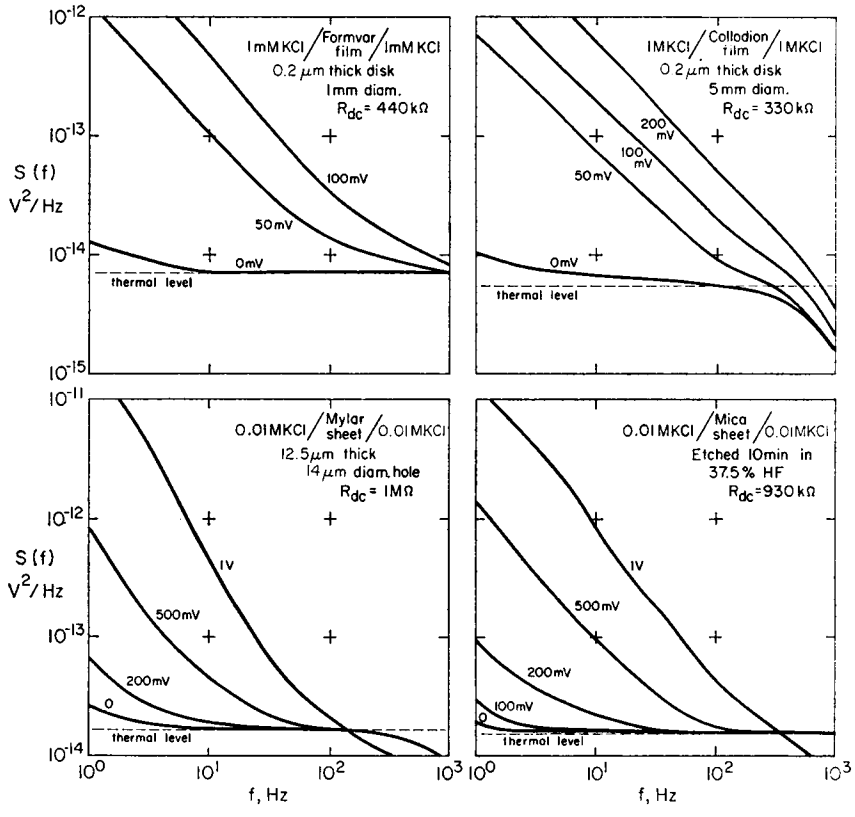

FIGURE 2 Power density spectra of fluctuations in electrical potential across four different types of porous synthetic membranes.

separated by porous membranes (pore diameters less than 30 μm) of etched sheets of mica (Bean et al., 1970), Formvar, collodion, and single holes in Mylar sheets show noise in excess of thermal levels in all of these porous membranes (Fig. 2). In addition, Segal (1972, p. 1380) states that ouabain had no effect on the noise spectrum of untreated skins. Assuming that metabolically passive currents in frog skin exhibit the same kind of noise as the synthetic membranes (Fig. 2), how does one conclude that the observed noise is associated with active transport?

The points which we have raised do not exclude either the possibility that excess noise can be observed in frog skin or that it is related to active transport; however, they do suggest that the specific measurements described (Segal, 1972) are too uncertain to draw any conclusions about the source of noise in frog skin.

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Damage and Repair of DNA in 5-Bromodeoxyuridine-Labeled Chinese Hamster Cells Exposed to Fluorescent Light

Dear Sir:

In our previous paper (1), we estimated the number average molecular weight (M_n) of DNA from Chinese hamster cells sedimented in alkaline sucrose gradients using the equation $M_n = 0.6\ M_w$, where M_w is the weight average molecular weight. The correct relationship should have been $M_n = 0.5\ M_w$. This correction does not apply to an earlier paper (2) involving these techniques where M_n was calculated directly by a fraction-by-fraction summation (3).

We regret any confusion this error might have caused. We emphasize, however, that since our results involved relative yields of single-strand breaks, the conclusions derived from them are not affected. To apply this correction, in Figs. 2, 5, and 6 M_n for single-stranded DNA from untreated cells should simply be 1.7×10^8 instead of 2×10^8 daltons as stated.

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