$$a_{cj} = a_{oj}\, 10^{(-Z_j E_{co}/58)}$$

The calculated activity of calcium ions in cytoplasm is again impossibly high.

It is true, as Prof. Briggs has said, that the results for calcium give no information as to the mode of entry of calcium into the cell. From the striking absence of equilibrium, I concluded[1] only that a plasmalemma of limited permeability to calcium was most probably present. As reference to the earlier communication will show, the suggestion of a lipid plasmalemma containing ion carriers was made to reconcile the following results obtained on *Nitella*: (*a*) radioactive cations appear to cross the plasmalemma much more rapidly than the tonoplast[4]; (*b*) the plasmalemma has a much higher electric resistance than the tonoplast[1].

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[1] Walker, N. A., *Nature*, **180**, 94 (1957).
[2] Briggs, G. E., *Nature*, **180**, 1142 (1957).
[3] Walker, N. A., *Austral. J. Biol. Sci.*, **8**, 476 (1955).
[4] Brooks, S. C., *J. Cell. Comp. Physiol.*, **38**, 83 (1951).

My statement, quoted by Mr. Walker, would be a *non sequitur* if "impermeable to cations" could be said not to imply permeability to anions. It is incorrect only if the alternative, the conductivity is independent of the concentration of anions, is correct. Mr. Walker can scarcely be maintaining this alternative.

I ought to have made it clear explicitly that I was referring to ohmic resistance both in my statements and in my calculation of the concentration of anion from the resistance of the cytoplasm. There would be a polarization resistance in the cytoplasm as well as that in the tonoplast. Mr. Walker's communication implies only the latter.

Mr. Walker has now provided more convincing evidence that the cytoplasm of *Nitella* and the external solution equilibrate very slowly with respect to calcium.

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Slide Gel Diffusion Precipitin Test

A modification of the plate gel diffusion test[1] is proposed, carried out on microscope slides. The use of a thin layer of media on the slide, together with a reduction in the distance between antigen and antibody basins, resulted in an earlier appearance of the reaction.

Ordinary new microscope slides were cleaned and polished with methyl alcohol and inspected for freedom from scratches, etc. Two lines, 4 cm. apart, were drawn on a slide with a grease pencil (alternatively two thin strips of zinc oxide tape were used) to limit the spread of the medium on the slide and thus obtain a reasonably constant depth of medium (approximately 0·16 cm.). This left 1½ cm. at one end of the slide which accommodated a type-written zinc oxide label identifying the individual slide. The bottom part (2 cm.) of the slide was left free for handling during reading.

Stock medium for this technique was the same as that used formerly[1]. Small quantities, stored in universal bottles with screw caps placed in a water bath at 64° C., were renewed weekly, thus avoiding the necessity of either preparing media in small quantities or remelting already congealed media. The melted media were distributed with a Pasteur pipette in 1-c.c. amounts between the two lines.

The cutting instrument consisted of a small 'Perspex' sheet in which the hexagonal pattern of two precipitin units were drilled, the distance between one basin and another within the same unit being 0·15 cm. The 'Perspex' sheet was made to fit over the prepared slide. Basins were cut by pushing a stainless steel tube, possessing a sharp edge, through holes drilled in the 'Perspex'. Prepared slides, which were kept in Petri dishes, were used preferably within a day or two, but they could be used after a longer period (1 week) if kept at 4° C.

The reagents were placed in the basins using a specially prepared small-bore pipette, as was done in the plate test. For convenience a pipette was fixed in the cap of a 10-c.c. bottle containing the specific serum, and in most cases where antigen was used as a suspension (for example, *Brucella*, *E. rhusiopathiae*, or an exudate such as myxoma). Slides in Petri dishes were incubated at 37° C.

The results were read and recorded as in the plate technique[1]. It was noticed that when slides were used the results were better when read as soon as the reactions were expected to appear, depending on the particular antigen–antibody system used. If slides were left for a longer period before being read some of the lines became blurred. It was possible by this method to demonstrate the precipitin reaction in a shorter time than when plates were used. Table 1 shows results obtained when the same reagents were used with both techniques at the same time. Four known viral and three bacterial antigen–antibody systems were used and the results shown were based on thirty-eight identical exposures of each antigen and its antibody.

An example of the slide technique is shown in Fig. 1. The slide on the left shows the result obtained after being left 18 hr. at room temperature, whereas that on the right shows the result obtained 90 min. after preparation of the slide. With each unit comprising six basins and each basin numbered in a clockwise direction 1 to 6 commencing at 12 o'clock,

Table 1

Antigen–antibody system	Plate		Slide	
	Minimum time of appearance	Maximum time of appearance	Minimum time of appearance	Maximum time of appearance
Viral:				
Canine distemper complex	5 hr.	Overnight	1 hr.	3 hr.
Canine hepatitis or Rubarth's disease	7 hr.	Overnight	3 hr.	4 hr.
Myxomatosis	1 hr. 50 min.	5 hr.	45 min.	1 hr.
Swine fever	3 hr.	Overnight	1 hr. 30 min.	2 hr. 10 min.
Bacterial:				
Brucella melitensis	5 hr.	Overnight	1 hr. 25 min.	2 hr. 30 min.
Cl. welchii D.	3 hr.	Overnight	1 hr. 40 min.	3 hr.
E. rhusiopathiae	5 hr.	Overnight	1 hr. 25 min.	3 hr.