Table 1. Optical transmittance of scintillating gels of aluminum stearate. I, transmitted radiant power; I_o, incident radiant power. The absorbance $A = abc$, where a is the absorptivity, b is the thickness of the cell, and c is the concentration in grams per liter.

Al stearate concn. (g/lit)	5000 A		4500 A	
	I_o/I	log I_o/I	I_o/I	log I_o/I
10	1.015	0.0064	1.029	0.0123
30	1.068	0.0286	1.092	0.0382
50	1.098	0.0407	1.128	0.0522
70	1.138	0.0561	1.178	0.0712

decrease would be inevitable if the discriminator bias were set higher in order to further reduce the background contribution for less active samples.

The variation of counting efficiency with total weight of precipitate at a constant specific activity is shown in Fig. 2. The samples were prepared from a fixed volume of active Na_2CO_3, mixed with varying volumes of inactive Na_2CO_3 of the same molarity. For this experiment a total activity of 0.05 μc was maintained in each sample. The counting data were obtained on a Dumont K1295 2-inch photomultiplier tube. The counting efficiencies dropped from 75.6 percent for 1 percent by weight of precipitate to 37.2 percent for 7.5 percent by weight of precipitate in these tests. The absolute counting efficiencies quoted are based on the stated activity of the Na_2CO_3 solution used, but the indicated accuracy of this source was ± 10 percent, and the calculated efficiencies may be in error by this amount. The effects of particle size and density were not investigated, but it seems obvious that settling and self-absorption effects would be reduced if a material of lower density than $BaCO_3$ (density 4.4) were used.

Some gels were formed in evacuated cells in order to eliminate the effects of oxygen quenching (*9*). An increase in pulse height and in counting efficiency was observed under these conditions. For example, the count of a sample containing 0.1665 g of $BaCO_3$ and 0.05 μc activity increased from 1360 to 1585 count/sec.

The optical transmission of the gels was also investigated. The thickened systems used by White and Helf (*7*) appeared to have poor transmittance as judged from the published photographs of their samples. The transmission characteristics of aluminum stearate gels were measured with a Beckman DK-1 recording spectrophotometer using 1-cm quartz cells. The data at 5000 and 4500 are shown in Table 1.

The data of Table 1 yielded a linear Beer's law plot whose slope gave a value for the absorptivity a of 0.00082 lit/gm-cm at 5000 A and 0.00104 at 4500 A.

Settling effects were negligible after the first day. A sample containing 0.05 g of $BaCO_3$ gave identical results within experimental error after 3 weeks' standing.

These counting experiments indicate that the scintillating gel technique provides a simple and effective means of assessing the activity of beta and alpha emitters. High sensitivity, good reproducibility, and linearity of response with activity are observed over a useful range of suspended material. The method compares favorably with the new technique recently described by Passman, Radin, and Cooper (*4*). In the same scintillator volume, about 7 times as much carbon can be incorporated as by their CO_2 method. The counting efficiencies appear better than those recently reported by White and Helf (*7*) for scintillating gels. This may be due to the superior optical properties of the gels reported here, but much of the difference is undoubtedly due to the wider gate settings used in our discriminator. In comparison with other techniques for assaying C^{14}, (*10*), scintillating gels offer many advantages. The samples are readily stored, vacuum manifolds are not needed, and compounds can be measured directly without the necessity of combusting or destroying the sample. The over-all sensitivity is superior to that obtainable with most gas counting techniques, and pulse height discrimination can be employed when one is counting radiation of differing energy (*11*).

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Potentially Simple Technique for Rearing "Germ-Free" Fish

"Germ-free" animals, those deprived of detectable microorganisms, are highly useful for studies in nutrition, disease, infection, and immunity. Investigators who have felt the need for this type of animal for the past 50 years have encountered many difficulties because rearing germ-free animals is a laborious and complicated procedure. Many complex techniques are involved, the necessary equipment is elaborate and costly, and the animals require careful and constant attention (*1*).

In the course of some experiments on the role of oral incubation in the cichlid fish, *Tilapia macrocephala*, L. R. Aronson and I (*2*) discovered that dipping fish eggs into a 0.04-percent formaldehyde-aquarium water solution for 10 minutes resulted in germ-free eggs which, when transferred aseptically to sterile water, grew into germ-free fry. Of 25 trials (consisting of 10 eggs per trial) treated in this manner (*3*), eight trials remained germ-free died during the experiment, Those embryos which did not remain germ-free died during the experiment, since certain of the bacteria proved to be lethal contaminants (*2*). The germ-free embryos, however, survived for 2 to 3 weeks after hatching, using the large quantity of yolk as their source of food. When these fry eventually died of starvation after the yolk was absorbed, they did not disintegrate. Some of the dead fish were kept as long as 4 months, when they were discarded. No microorganisms could be cultured from a number of the dead fry when culture was attempted in Difco nutrient broth, Difco nutrient agar, and thioglycollate media. Likewise, no mold and fungal growths were detected.

Since this problem is not related to the work of the department of animal behavior, it was not pursued, but the observations suggest that *Tilapia macrocephala* could be fed aseptically and brought to germ-free maturity in an appropriately equipped bacteriological laboratory with fewer of the complications that are encountered in other forms. For example, Baker and Ferguson (*4*) found

that germ-free platyfish, *Xiphophorus maculatus,* required live food for their maintenance. This caused many technical difficulties and led to inadequate growth. *Tilapia macrocephala,* on the other hand, are regularly raised on dried food, which can be sterilized easily, and preliminary tests indicate that the dried food does not lose its nutritive properties when it is autoclaved. In addition, fertile eggs are available in large numbers, for the adults reproduce during the entire year at frequent intervals. Many fish can be raised together, they require little care, and the environment can be kept reasonably uniform. What is still needed is a simple device whereby the fish can be fed and whereby gases can be exchanged aseptically.

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Concerning the "Cellularity" or Acellularity of the Protozoa

Most protozoologists (*1*) probably will agree with Alan Boyden's recent contention (*2*) that it is logical to consider protozoa as "cellular" organisms (that is, cells). In fact, over 40 years ago, Minchin (*3, 4*) defended this notion well, shortly after the appearance of Dobell's vigorous attack (*5*) on the cell theory and its implications concerning the nature of protozoa. However, the recent outburst of criticism directed at Dobell and Hyman (*6*) by Boyden seems rather harsh and narrow. Neglected entirely, furthermore, was consideration of the fundamental question of how, precisely, a cell may be defined; until an entirely satisfactory answer to this question is available, it may be as improper to insist dogmatically that protozoa are unicellular as to claim that they must be noncellular. It is the purpose of the present comment to show that the whole problem is considerably more complicated than Boyden has indicated.

C. G. Ehrenberg (*7*), many years ago, championed the notion that protozoa are "vollkommene Organismen." But, as a consequence of F. Dujardin's exposure (*8*) of the fallaciousness of his contemporary's *morphological* observations, many biologists came to think of the protozoa as simple and as comparable with a single metazoon only when the physiologic (or sexual) life cycle of an entire clonal population was considered. In the light of the atmosphere of the period in which Dobell wrote his trenchant essay, he deserves credit for focusing fresh attention on the fundamental truth of Ehrenberg's idea: in general a *single* protozoon is as capable of independent locomotion, feeding, growth, reproduction, regeneration, and so on, as is any *entire* metazoan organism.

Neither Dobell nor Hyman directly denied the essential homology of nuclei and various cytoplasmic structures that are possessed in common by metazoan cells and the protozoa, since they admitted that the same fundamental organization is to be found in members of both groups. In their insistence that an individual protozoon is, also, homologous with an entire multicellular organism, I think perhaps the only serious breach of logic is the use of the word *homologous* (which is employed directly in this connection, incidentally, only by Dobell). As Minchin (*4*) observed, "the view generally held that the entire organism of a Protozoon is truly homologous with a single body-cell of a Metazoon seems to me quite unassailable. . . . On the other hand, any Protist, as an organism physiologically complete in itself, is clearly analogous to the entire individual in the Metazoa—a comparison, however, which leaves the question of genetic homology quite untouched."

What is a cell? Although it would be inappropriate to offer a lengthy treatment of the question here, we must consider the matter to some extent. Dobell added to the well-known classical definition of the cell the qualification that it "is a part of an organism and not a whole organism." Thus, his insistence that protozoa are noncellular represents a stand not at all inconsistent or illogical, it seems to me, with respect to his own definition of a cell. Minchin himself, Dobell's most outstanding critic, suggested (*4*): "So long as the Protozoa are studied entirely by themselves, without reference to any other forms of life, they may be termed non-cellular in the sense that they are not composed of cells." Hyman also described a cell as "one nucleated division of an organism" (but compare *9*). One must acknowledge that adherents to the definition (be it good or poor) proposed by Dobell and Hyman are placed in an uncompromising position: protozoa are not parts of organisms and thus cannot be cells. The several workers (for example, Lwoff, *10*) on the physiology of protozoa who presumably adopted the acellularity concept may well have been accepting the spirit, only, of Dobell's interpretation in order to emphasize the striking similarities in the biochemistry of the individual protist and the entire metazoan animal.

Nearly a decade ago, J. R. Baker (*11*) criticized Dobell's ideas quite strongly, yet he offered an original definition of a cell ("a mass of protoplasm, largely or completely bounded by a membrane, and containing within it a single nucleus formed by the telophase transformation of a haploid or diploid set of anaphase chromosomes") which, on his own admission, obliges one to consider all "polyenergid" protozoa, including the ciliates, as noncellular organisms. To follow Baker, one would have to recognize both unicellular and acellular forms; the distinction would be dependent solely on the number of nuclei present.

Some biologists have suggested that certain protozoa are truly multicellular in their organization. G. S. Carter's discussion (*12*) is particularly pertinent: he calls attention to the cnidosporidian *Myxobolus,* in which several somatic cells are observable at one stage in the life cycle, endowing the organism with genuine multicellularity.

Thus, it need not be considered altogether illogical to think of the protozoa as comprising a variety of forms some of which are clearly only unicellular, others multicellular in certain stages, still others acellular throughout their lives. Personally, however, I favor rejection of the circumscribed definitions of a cell offered by both Baker and Dobell, and I consider the protozoa, as a group, to be unicellular organisms (not necessarily animals). But the dangers associated with such a generalization should always be kept in mind. A single protozoon is a whole individual—more than the equivalent of a component, dependent part (cell) of a highly integrated multicellular organism. In spite of its morphologic homology with a dissociated cell of the metazoan body, it often possesses an unparalleled degree of subcellular differentiation. Physiologically, as well as morphologically, the majority of the protozoa are independent, complex organisms, far from simple in spite of their typically microscopic size.

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