In answering the question "Are there any 'acellular animals'?" in the negative, Alan Boyden (*1*) refers to the cell theory as one of the greatest generalizations of biology. Certainly it has been, and is, of the utmost importance in the very extensive areas where it is relevant. But any successful generalization carries within itself the potential capacity to become a dogma. When that happens, it may well serve as a Procrustean bed into which unhappy facts, if they do not fit properly, must be forced by violence. I wish to call attention to one fact of organization that can be fitted into the cell theory only by such violence.

A large myxomycete plasmodium may contain millions of nuclei imbedded in a common matrix. At its advancing margin, such a plasmodium is essentially naked, bordered only by an ectoplasmic layer from which delicate pseudopodia may extend. The remaining portions, and particularly the veins that are usually its most conspicuous elements, are surrounded by a thick, gelatinous, enucleate sheath, within which the enclosed nucleate protoplasm circulates, and which collapses and is left behind on the substratum as the plasmodium advances. How may such a structure be forced into the cell theory? Two attempts have been made.

One, repeated many times, refers to such a mass as a single, multinucleate cell. As anyone who has cultured plasmodia knows, it may break up into two or a dozen smaller plasmodia, which may combine again in any possible degree. A large plasmodium, cultured in a Wardian case, may cover an area of half a square meter; there is good evidence that, in nature, it may be much larger. If this is to be regarded as a single cell, it must carry with it into the cell theory the concept of an enormous cell that may break up into numerous smaller, but still relatively enormous, cells that may re-fuse into larger units in a sequence that may be repeated an indefinite number of times until it is interrupted by fruiting. To call such a structure a cell seems to go far toward destroying the usefulness of the cell theory in areas where it is really important.

Another suggestion, less popular but not infrequently invoked, is that a myxomycete plasmodium is a multicellular organism in which each cell is represented by an individual nucleus and that portion of the surrounding cytoplasm that may be assumed to be under its influence at any particular instant. Examination of the flow in a plasmodial vein under a moderately high power objective demonstrates that there is no constant relationship between a nucleus and its surrounding protoplasm. This is evidenced by the relative movement of nuclei and visible extranuclear granules borne in the same stream. Such an explanation, then, is purely idealistic and quite completely divorced from observable fact.

It is, of course, recognized that the swarm-cells, which often function as gametes, the zygotes, and the spores of Myxomycetes may legitimately be regarded as cells. But this does not affect the argument for regarding the organization of plasmodia as acellular. Such recognition, it is true, carries further implications. If plasmodia are to be called acellular, what is to prevent one from taking seriously the arguments that have been advanced with respect to the lack of cell organization in mucors and numerous other fungi? Discussion of this problem goes beyond the scope of these comments, but its existence should be noted.

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Reference

1. A. Boyden, *Science* **125**, 155 (1957).

4 February 1957

I am pleased that my simple question, "Are there any 'acellular animals'?" has resulted in the expression of opinions both favorable and unfavorable, because there are larger issues involved than the immediate answer to the question. My brief report and the three replies could provide an interesting study in biological thinking.

J. O. Corliss agrees with me in considering the protozoa, as a group, to be unicellular organisms. "But the dangers associated with such a generalization always should be kept in mind." My view is that the dangers of denying this generalization are far greater than those of accepting it. For this is primarily a question of homology, and if, as is generally admitted, the body of the individual protozoan corresponds, part for part, with the individual cells of metazoa, then we must refer to them all as "cellular" (or as "acellular"). The history of our terminology is such that we are required to use the term *cellular* for such protoplasmic organization, and a decent respect for our heritage of words would confirm us in this usage.

It is granted that precise definitions are desirable, but the trouble is that nature presents us with so many variations on a central theme that precise definitions are difficult. The more important capacity is to recognize the central theme —in this case, the general pattern of protoplasmic organizations commonly referred to as "cellular." Only a fundamentally different pattern of organization should be called "acellular," but there is no such difference in organization between the gametes and zygotes, between the zygotes and subsequent stages of metazoa, or between any of these cells and individual protozoa.

The matters raised by S. H. Hutner and L. Provasoli are important in themselves, but I do not agree with them in the belief that progress in the understanding of problems of differentiation and integration requires us to consider protozoa acellular or to believe that cellular stages arise out of acellular stages in metazoan ontogeny. All protoplasmic systems are organized, and there is surely some virtue in accepting this fact as we search for the new truth.

As to the points raised by G. W. Martin, they do not, in my opinion, destroy the validity of the generalization that animals are cellular. There may always be differences of opinion in regard to whether certain plasmodia are unicellular or multicellular, but they remain *cellular* for all that. If fundamentally different kinds of protoplasmic organization in animals should be found, they might become of great interest from the standpoint of primitive evolution, but the cell theory would still stand.

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13 March 1957

Magnetometer Method for Recording Gastric Motility

Although human gastric motility has been recorded for many years, all accepted methods to date, other than roentgenographic, require the subject to swallow the sensing element with its attached tube or wires issuing from the mouth or nose. These leads constitute a marked and relatively persistent noxious stimulus. One of us (M. A. W.) has searched for many years for a practical, bland technique for continuous remote recording of stomach action. With the assistance of many people (*1*) we have devised such a technique (*2*).

The subject needs only to swallow a magnet about the size of a vitamin capsule and then recline on a cot. A detector placed beneath the wooden cot about 18 inches below the subject's stomach senses the field variations resulting from the magnet's presence and motion and translates them into electric variations which are then amplified and recorded. Although a program of direct validation by motion-picture fluoroscopy plus the simultaneous use of a gastric balloon is not complete, the frequencies and relative amplitudes of the recorded waves seem to correlate well with previous results.

Although there are a number of magnetometer and metal-detection systems available, the unit we first used was a Waugh magnetometer type W-2, which was produced during World War II to bench-check steel aircraft components for residual magnetism. It required minor modifications for working into the chopper input of an Offner model D electroencephalograph. The detector responds to the absolute intensity of a steady magnetic field in a particular direction and will register zero when oriented in a plane perpendicular to the direction of the ambient field. The system is calibrated by measuring the effect of rotating the pickup a given amount about a vertical axis from its null point with respect to the earth's field. The field to which the instrument is responding for calibration is presumed to be the product of the horizontal intensity and the sine of the angle of displacement. On the basis of the published value of 0.25 gauss (*3*) for the horizontal intensity of the earth's field in California, 0.2 mgauss/cm of pen travel seems to be ample over-all sensitivity, the periodic field variations with motility present characteristically approaching 1 mgauss peak to peak.

The magnets used are 3/16-inch diameter by ½-inch long General Electric Company sintered alnico-5 rods furnished cut to length. These magnets are not treated in any special way to increase or preserve the degree of magnetization they have when received. They are prepared for ingestion by coating with polystyrene. Subjects report neither awareness of them after ingestion nor any kind of after effect, and the data (*4*) indicate that the ingested magnet probably remains within the stomach of most subjects for periods of 2 hours or longer. The field variations recorded can be shown to be a function not only of periodic amplitude of magnet movement, but of mode of motion, orientation, and position with respect to the detector.

The method appears to have many uses beyond the recording of stomach motility. For example, it is currently being used to detect particular patterns of movements in small laboratory animals; the magnets are fixed under the skin. The Waugh type W-2 magnetometer has been modified and improved by the current manufacturers (*5*) and by William T. Kyle, Electronic Facility, College of Engineering, University of California, Los Angeles. Information concerning the improved circuit we now employ may be obtained from Kyle until it is published.

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References and Notes

1. Individuals too numerous to mention have contributed to discussions of this problem. John Dinning of the UCLA College of Engineering is to be credited with suggesting the successful solution.
2. This project was supported in part by USPHS grant M788.
3. U.S. Coast and Geodetic Survey data of 1911–12.
4. M. A. Wenger, B. T. Engel, T. L. Clemens, *Am. Psychologist* 10, 452 (1955).
5. Irwin Laboratories, 1238 S. Gerhartane, Los Angeles 22, Calif. E. M. Irwin holds U.S. patent No. 2418553 on the detector unit.

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Stigmasterol in Cigarette Smoke

The presence of phytosterols in tobacco leaf (*1, 2*), seed (*3*), and smoke (*4*) has been previously reported; in only one case was a structure assigned to the sterol, that of γ-sitosterol isolated from *Nicotiana tabacum* (*2*). We wish to report the isolation of stigmasterol, a compound whose melting point differs from that of the other sterols recorded, from cigarette smoke (*5*).

The smoke generated from regular size, American, blended cigarettes was fractionated as we have described earlier (*6*). Fraction M (200 g) was chromatographed on silica gel; a mixture of 84 percent benzene and 16 percent ether eluted material whose infrared spectrum had absorption bands in the 9 to 11-μ region similar to those of many sterols (*7*). These bands were used to guide further isolation work. Fractional recrystallization from 1/1 benzene and methanol was followed by three chromatographies on acid-washed alumina, fractional recrystallization from 4/1 methanol and ethanol, and recrystallization from 1/4 benzene and petroleum ether (30° to 60°C), whereupon 35 mg of colorless crystals, mp 165.0 to 168.5°C, $[\alpha]_D^{20}$ – 40.2° (*8*) was obtained (stigmasterol, mp 170 to 171°C, $[\alpha]_D^{17}$ – 45.8° (*9*). The compound gave a positive Liebermann-Burchard test, and a precipitate with digitonin; it absorbed 2.0 moles of hydrogen (*10*) (assuming a molecular weight of 411); it exhibited a "false energy" maximum (*11*) at 206 mμ in the ultraviolet, ruling out the possibility of conjugated double bonds; and it had an infrared absorption spectrum consistent with that of stigmasterol. The reduction product had a melting point of 136.8 to 137.3°C $[\alpha]_D^{20}$ 25.3° {stigmastanol, mp 137°C (*12*), $[\alpha]_D^{20}$ 24° (*13*)}; its acetate melted at 131.2 to 132.4°C and had a rotation of $[\alpha]_D^{20}$ 16.2° {stigmastanyl acetate, mp 129.0 to 129.5°C $[\alpha]_D^{20}$ 15.4° (*14*)}; and its benzoate had a melting point of 135.0 to 136.0°C $[\alpha]_D^{20}$ 19.7° {stigmastanyl benzoate, mp 135.0 to 136.°C $[\alpha]_D^{20}$ 19.6° (*15*)}.

The acetate and the benzoate of the original sterol were prepared; the former, after several recrystallizations from 4/1 methanol and benzene, had a melting point of 140 to 141°C and a rotation of $[\alpha]_D^{20}$ – 51.9° {stigmasteryl acetate, mp 140 to 141°C $[\alpha]_D^{20}$ – 50.3° (*16*)}. It did not depress the melting point of an authentic sample of stigmasteryl acetate (*17*), and it had an infrared spectrum identical with that of the authentic material; the benzoate, after repeated recrystallization from 6/1 methanol and benzene, melted at 161.0 to 161.5°C $[\alpha]_D^{20}$ – 24.3° {stigmasteryl benzoate, mp 160.5 to 161.5° $[\alpha]_D^{20}$ – 24.5° (*18*)}.

The crude steroid fraction from fraction M contains other steroids which we have not as yet isolated in pure form. A sterol has also been obtained from fraction K (*6*); the data are: mp 149.5°C $[\alpha]_D^{20}$ – 63°, positive Liebermann-Burchard test; precipitate with digitonin; acetate, mp 137.0 to 138.0°C; benzoate, mp 124.5 to 125.0°C. The quantity isolated was too small for us to effect rigorous purification, and these values may be open to revision.

Khanolkar, Panse, and Divekar (*2*) found the sterol glycoside which they isolated from tobacco leaf to be readily hydrolyzed by dilute hydrochloric acid. The possibility therefore exists that, inasmuch as an acid extraction step was utilized in our fractionation of the cigarette smoke condensate, stigmasterol may be present in smoke in part, or in whole, as a glycoside. The quantity of sterol isolated corresponds to 0.5 μg per cigarette; this figure must be taken only as a lower limit inasmuch as considerable losses were sustained in the isolation process.

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References and Notes

1. F. T. Mosca, *Gazz. chim. ital.* 43, (II), 440 (1913); M. Kobel and C. Neuberg, *J. prakt. Chem.* 143, 29 (1935) (saponins); A. Shmuk, *Vsesoyuz. Inst. Tabach. i Makhoroch. Prom.* 133, 3 (1937); *Chem. Abstr.* 32, 4723 (1938) (fermented tobacco).
2. V. R. Khanolkar, T. B. Panse, V. D. Divekar, *Science* 122, 515 (1955).
3. C. Venkatarao *et al., J. Indian Chem. Soc.* 20, 374 (1943).
4. O. Schürch and A. Winterstein, *Z. Krebsforsch.* 42, 76 (1935).
5. We are indebted to the American Cancer Society for a grant in support of this work.
6. A. I. Kosak, J. S. Swinehart, D. Taber, *J. Natl. Cancer Inst.* 17, 375 (1956).
7. K. Dobriner, E. R. Katzenellenbogen, R. N. Jones, *Infrared Absorption Spectra of Steroids* (Interscience, New York, 1953).
8. All rotations reported in this paper, whether taken from the literature or current work, were run in chloroform.
9. J. C. E. Simpson and N. E. Williams, *J. Chem. Soc.* 1937, 737 (1937).
10. Hydrogen uptake was rapid when a 5-percent rhodium on alumina catalyst was used but very slow in the presence of a platinum catalyst.