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EDITORS' PREFACE

The environment can alter the properties of a population of micro-organisms in two ways: either through genetic variation, following mutation or selection, or through non-genetic variation, that is, by producing changes in behaviour unaccompanied by alterations in genetic structure. The second effect forms the chief subject of this volume which contains the papers to be discussed at the Eleventh Symposium of the Society for General Microbiology.

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Let dogma eat dogma

DON MARQUIS: *The Almost Perfect State* (1927)

As we uncover the rich microbiological stew that is this Symposium, I propose to add a dash of anthropocentric seasoning.

With the Darwin centennial in mind, we may ponder on the microbial origins of the devices by which man responds to the environment. That this responsivity somehow rests on animality—more narrowly, meta-zoan animality—with like obtuseness is neglected by biologists and deplored by theologians. Worse, man talks like an *arriviste*: in Western culture 'animal' is a taunt, and the distinction of animals, phagotrophy, has a pejorative connotation, as in our opening quotation (a demonstration that phagotrophy so inescapably colours our thinking that scientific method itself is expressible in terms of phagotrophy).

Darwin, no snob, was 'ardently theriophilous', delighting in 'the excellence of every living thing' (Fitch, 1959). Thanks to this spirit surviving in protozoologists, several different kinds of protozoa are in pure culture: material is at hand for a decently comprehensive bio-chemistry of animals.

MEMBRANE-FORMATION AND PREY-CATCHING

Huxley (1876) defined animals as eaters. More elaborately, Haldane (1954a) described animals as wanderers seeking fuel and ready-made spare parts; if one adds 'characteristically ingested in chunks' one has a workable primary definition. That certain phytoflagellates which, like *Euglena*, are non-ingesters, are treated in texts on protozoa and in zoology courses has practical justification: some euglenoids are skilful animals; seemingly, protozoologists, in laying out their academic domain, have indulged in a predation rivalling that of some of their pets.

My immediate object is to consider the question: Wherein do the growth-responses of protozoa offer homologies to human responses?

Conversely, how do metazoan responses illuminate protozoan animality? Since, for predators, growth and behaviour centre on capture, digestion, and assimilation of prey, behaviour and growth in them are intimately joined, and so one may find a hormone for one organism a conventional growth factor for another. Some examples will be mentioned later.

For a unicellular predator to tell self from prey—a function of the apparatus of immunity—it must compartmentalize itself, if but to guard its deoxyribonucleic acid from insidious usurpers such as the temperate bacteriophages (Jacob, Schaeffer & Wollman, 1960). (One wonders whether the absence of phage-like viruses in protozoa, related algae, and fungi denotes a phylogenetic immunity conferred by envelopment of the nucleus in a membrane, or merely inadequate technical methods.) The quintessential specialization of Protozoa, and of phagocytic cells generally, may be synthesis, on cue, of a special membrane—the food-vacuole membrane—along with digestive enzymes and expanded excretory organelles.

Membrane-making takes work, as inferred from the heightened O_2 consumption and diminution in intracellular glycogen of leucocytes ingesting bacteria or inert particles (Becker, Munder & Fischer, 1958; Elsbach, 1959; Karnovsky & Sbarra, 1960). The latter workers noted also increased uptake of acetate and phosphate, interpreted as reflecting the rich lipid content of the membranes. In keeping, furthermore, glycolysis inhibitors stop phagocytosis in polymorphonuclear leucocytes, and use of exogenous glucose increases during phagocytosis (Cohn & Morse, 1960).

Membrane-formation is spectacular in the slime mould *Physarum polycephalum* (Stewart & Stewart, 1960). On cutting a vigorously streaming plasmodium, a globule wells out and sets firm, soon becoming a viable independent plasmodium. Also on slow desiccation 'the non-cellular, continuous sheet of fluid protoplasm divides into a brittle mass of small cell-like structures...increasing tremendously the total area of surface membrane'.

Many ciliates regenerate well. Thus fragments of *Tetrahymena pyriformis* as small as thirds may regenerate (Albach & Corliss, 1959). Since ciliates are commonly under high internal pressure, a healing membrane must form fast or the animal bursts. *T. pyriformis* is easily grown in chemically defined media, which opens the way to close analysis of conditions for regeneration.

Indiscriminate, i.e. cue-less, gluttony seems allied with prodigies of membrane-formation. The monstrous multinucleate amoeba *Pelomyxa palustris* eats everything in its path (Kudo, 1960). Feeding is by posterior

viscous villi; when body movement is reversed, the new posterior end develops villi. Multiplication by other than simple division has not been observed nor have the complicated reproductive structures met in Acrasieae and slime moulds; it may be that in the large amoebae obvious structural complexity has been sacrificed to membrane-formation.

Roth (1960) traced formation of many small vesicles ($0.2-0.5\mu$ dia.) at the cytoplasmic side of the food-vacuole membrane. He concluded that micropinocytosis (ingestion of fine droplets) mediated transfer of the contents of food vacuoles into the cytoplasm and calculated that, starting with $20 \times 10^3\mu^2$ of surface area of food vacuole, over the 720 min. required for digestion each μ^2 of original vacuole membrane duplicated its surface area every minute. To account for pinocytosis allowing passage into the cytoplasm of substances which cannot diffuse across the plasmalemma, he postulated progressive changes in the membrane. One such change might be increased acid phosphatases— a change especially striking in rat macrophages ingesting erythrocytes in Kupffer cells of liver, and in *Amoeba proteus* (Novikoff, 1960). (*A. proteus* should not be called, according to Kudo, 1959, '*Chaos chaos*'.) Kudo notes that in no large free-living amoeba has morphological change been correlated with environmental or internal conditions.

The cues for pinocytosis in *Amoeba proteus* are rather non-specific: simple cations, rabbit gamma-globulin, gelatin and glutamate (reviewed by Holter, 1959 *a, b*). Marshall, Schumaker & Brandt (1959), generalizing from the binding of lysozyme by homogenates of *A. proteus*, suggest that pinocytosis begins with binding of proteins or other inducers to the cell surface, the essential groups of the inducer carrying a positive charge—a theory supported by the strong attachment of toluidine blue to the surface of *A. proteus* and its ability to induce pinocytosis (Rustad, 1959).

Another specialization for phagocytosis is a sticky surface or at least one binding protein. It is as disconcerting to a naïve sense of the phyletic fitness of things to see an amoeba capturing ciliates as to see a turtle catching ducklings or a spider snaring humming birds. Stickiness is in some way associated with fine fibrous extensions of the plasma-lemma in *Amoeba proteus* and *Pelomyxa carolinensis* (Pappas, 1959); how the posterior villi of *Pelomyxa*, previously mentioned, help stickiness is not clear.

The relation of 'lysosomes', widely distributed bodies having single membranes and high in acid phosphatases (Novikoff, 1960) to phago-cytosis and pinocytosis generally is a new subject (de Duve, 1960).

Amoeba proteus is so seductive a research object that the non-

microbiologist is apt to be shocked to learn it is not in pure culture. It can be reared fairly dependably on *Tetrahymena* supplemented with bacteria and yeasts. It is easily freed of other organisms; the problem is finding a non-toxic soluble medium (Prescott, 1959). Some small soil amoeba of the *Hartmannella* type do grow in autoclaved peptone media; the minimal nutritional requirements of one include thiamine, vitamin B_{12}, and some still-unsorted amino acids (Adam, 1959); dependence on carbohydrate and utilization of acetate has been demonstrated in a similar form (Band, 1959). What induces the stage in the 'life cycle' of '*A. proteus*' marked by sporulation and the hatching out of small amoebae (reviewed by Jepps, 1956), is unknown.

GROWTH FACTORS AND MICROBIAL ECOLOGY

Cue-less gluttony may prevail in the tiny (down to 1 μ) photosynthetic chrysomonads such as those beautifully figured by Parke, Manton & Clarke (1955, 1959) who fed them colloidal graphite to observe phagocytosis. Knight-Jones (1957) supposes that speed is one of their nutritional specializations; μ-flagellates, especially abundant in warmer seas, '. . . dash about. . . squandering the energy which they can get from sunshine, their movements and relatively large surface area presumably helping them to find and mop up the sparse nutrients which they need just for growth'. This is an exceedingly successful way of life: Knight-Jones (1951), citing results by H. W. Harvey and his own, notes that 90 % of the plankton passed through a 40–50 μ mesh net consisted of flagellates. Indeed, μ-flagellates may be the most abundant organisms on earth: the waters around the British Isles harbour ~ 1000/ml.

A litre of sea water may hold a hundred different flagellates and diatoms. Predilections for different growth factors, joined with differences in efficiency of absorption of minerals and use of light, may underlie the succession of species in natural waters and the occasional wealth of forms (Provasoli, 1958a). Fine subdivisions of ecological niches are set up by differences in utilization of congeners of vitamin B_{12} by various micro-organisms (Droop, McLaughlin, Pintner & Provasoli, 1959). In marine littoral diatoms, variations in respect of obligate photoautotrophy and heterotrophy, superimposed on variations in auxotrophy (thiamine and cobalamins), reveal more modes of subdivisions of environments (Lewin & Lewin, 1960). Superposition of variations in ability to digest microbial bodies upon graduations in efficiency in trapping light define still more niches.

Work with non-protozoan micro-organisms and metazoa provide

some working hypotheses for sorting out the specialties of protozoa. The soil microflora is a consortium of chemical specialists. Bacteria sorted out as to growth requirements (Lochhead, 1958) are gradually being matched up with those isolated from enrichment cultures with rare substrates. That the growth of some seaweeds may depend on environmental factors also operating on protist and other groups which include prototrophic and auxotrophic members is suggested by the cobalamin requirement of the red alga *Goniotrichum elegans* (Fries, 1960). As in plant tissue cultures where hormones often serve as exogenous growth factors, the lines between hormones and growth factors are blurred in the green marine alga *Ulva lactuca*: for normal development of the thallus, a sea-water culture medum needs supplementation with indoleacetic acid, kinetin, and gibberellin (Provasoli, 1958b). The *Ulva* situation is complicated: sea water cannot yet be replaced by the usual synthetic substitutes, and some sea waters are inert (Provasoli, personal communication). A start has been made in identifying auxin-like substances in sea water (Bentley, 1959). If to these newly recognized specializations one adds variations in ability to assimilate microbial prey, scope for chemical specialization in protozoa is wide indeed. Man, on this scale of nutritional fastidiousness, viewed in the light of the Knight-Lwoff canon (see Snell, 1951) of loss of function in evolution, has boxed himself into an ecological corner: he needs one growth factor (vitamin B_{12}) primarily of bacterial origin (a reminder of a filter-feeding past? Berrill, 1955); the auto-oxidizable ascorbic acid, apparently absent from most microbes (except perhaps for photosynthetic bacteria); vitamin D, for which he needs light or other metazoa; and vitamin E, quite unstable, which, with ascorbic acid, restricts him to fresh foods. Inability to digest cellulose and most polysaccharides eliminates higher plants as a souce of fuel. And, finally, he requires sundry other lipid, poorly diffusible growth factors. Were man a protozoon, he would be described as a virtually obligate phagotroph: demanding a great variety of food (and so, obligately omnivorous), preferably in the form of fresh bodies, not carrion, still with their antioxidants. This nutritional complexity would strike the investigator as improbable if not downright irrational—the outcome of a tortuous gastronomic history. The difficulties in defining the growth requirements of carnivorous protozoa, e.g. *Amoeba proteus*, assume in this perspective a more understandable cast. The origin of the thyroid in the mucus-secreting particle-trapping endostyle of early chordates presents a striking demonstration that a mucoid food-catching membrane may have remarkable biological potentialities.

SYMBIOSIS

Ruminants have largely escaped the biochemical and ecological trap described for man by evolving into fermentation tanks—thereby helping man to survive in his trap. Likewise many protozoa and invertebrate metazoa have symbionts. Cultivation of the symbiotic zooxanthellae of coelenterates led to the proof (foreshadowed by a few purely observational data) that their zooxanthellae are morphologically primitive dinoflagellates (McLaughlin & Zahl, 1959), which adds to the likelihood that this symbiosis was anciently established. The symbionts of radiolarians and other marine non-coelenterate invertebrates may include such 'primitive' flagellates as cryptomonads (McLaughlin & Zahl, 1961). Quite likely invertebrates, notably corals, have sheltered, as in a museum, an array of primitive flagellates. Work on zooxanthellid lifecycles and the environmental control of host-symbiont relations, especially as influenced by light, has barely begun (Freudenthal, 1959).

METAZOAN ORIGINS, PREDATION, AND ENZYMIC VERSATILITY

The varied compositions of bacterial cell walls (Salton, 1960) implies that even the most versatile predator cannot utilize all microbial prey. Nanney (1960) supposes that an organism carries, for economy, not all useful enzymes, but the blueprints for the enzymes in its genetic library, and has evolved switches for turning on and off enzyme production; and that persistence of environmentally induced differences in microorganisms of the same genotype (exemplified by temperature-induced antigenic differences in *Paramecium* and induction of permeases in *Escherichia coli*) foreshadows the embryological differentiations of genotypically identical cells.

Naegleria gruberi, an amoeba, can grow a uroid (tuft of villi) at its rear when put in a 'more than usually watery' medium, and also anterior flagella which enable it to swim off (Willmer, 1960). Impressed by this apposition of amoeboid feeding and flagellate dispersal stages, and the prevalence of these cell types in the embryos of many invertebrates, Willmer thinks that the primitive metazoon might have been a symbiosis of 'saprobiotic' flagellate cells and phagocytic amoeboid cells. This theory is opposed to the theory of J. Hadži (see Hanson, 1958) deriving the metazoa from the cellularization of a large multinucleate ciliate, such as the ultra-voracious *Dileptus*.

PROTISTAN HORMONES AS PHYLOGENETIC MARKERS:
STRESS IN *OCHROMONAS*

With patent disagreement about how the metazoa arose, the search for phylogenetic markers continues. A conservative behaviour pattern bespeaks a conservative metabolic pattern, and conversely (Lorenz, 1960). Resemblances in mediators of behaviour—hormones—in metazoa and protozoa might reveal uniquely metazoan-like patterns, assuming that nicely archaic protozoa are extant. Hormones controlling intake and interconversion of foodstuffs ought to be especially deepseated in animals; in metazoa these processes are conspicuously under hormonal control. Zuckerman's (1952) idea that the adaptive reactions of an animal in response to changes in its environment are mediated through the nervous and endocrine systems, may be juxtaposed with Haldane's (1954*b*) idea that the hormones of multicellular animals originated in the intracellular signals of protozoa and later in the signals of neighbouring cells. The problem comes down to the cellular targets of hormones in metazoa and the origins of neurohumoral systems in invertebrates and protozoa. Developments in microbiology hint that matters may not be as bleak as Stettin (1959) makes out for metazoan hormones or Galston & Purves (1960)—to take a look at the metaphyta—for auxin. Thus acrasin, the aggregation hormone of *Dictyostelium*, is replaceable by the weakly active Δ^{22}-stigmasten-3β-ol, isolated from *D. discoideum* (Heftmann, Wright & Liddel, 1959), a finding not yet reconciled with the high activity of female sex hormones, e.g. oestrone sulphate is active at 0·05 μg./ml. (Wright & Anderson, 1958). This particular stigmastenol is probably active as a vitamin for *Paramecium* and the guinea-pig (Conner & van Wagtendonk, 1955).

A certain similarity in steroid relations attends the non-plasmodial *Labyrinthula* which streams, cell by cell, toward food, by an unidentified cell-to-cell signal, effective even among widely scattered cells (Aschner & Kogan, 1959). Some labyrinthulas have a steroid growth requirement. Vishniac (1957), seeking transformation products, fed labelled cholesterol (which satisfies the requirement) to *L. minuta* var. *atlantica*. The only steroid recovered was cholesterol itself.

Metazoan and metaphytan hormones presumably evolved from a common protistan source; the resemblances between indoleacetic acid and serotonin speaks in favour of this theory: zoological interest attends tracing the origins of metaphytan hormones. As tissue cultures of brown or red seaweeds—probably nearer to the metazoa than are the chlorophytes—are not yet available, we are thrown back on the growth

processes and behaviour of protozoa for evidences of the protistan origins of metazoan hormones, and perhaps of metaphytan hormones also. The contributions in the present Symposium on adaptive enzymes (see contributions by Pardee, Kerridge, etc.), bearing on cellular memory, might with equal justice be considered to shed light on the primordial molecular adjustments of behaviour to the environment. It is unfortunate that knowledge of adaptive behaviour in protozoa is so scanty as compared with bacteria and viruses, except for drug resistance—but here genetic analysis is lacking because of the lack of demonstrable sexuality in the investigated pathogens.

TEMPERATURE STRESS IN FLAGELLATES AS AN APPROACH TO ENDOCRINOLOGY

As one views the many failures to demonstrate interpretable responses in micro-organisms of metazoan hormones which regulate the main metabolic pathways, one wonders how best to arrange conditions for possible demonstration of such responses—if demonstrable at all. In metazoa, hormones regulate the interconversion of fat, protein, and carbohydrate: e.g. insulin affects utilization of glucose and synthesis of long-chain fatty acids; the corticosteroids gluconeogenesis from protein; and anterior pituitary hormones can induce ketosis (Krebs, 1960). The line we are following starts with the assumption that an omnivorous predator is adept at interconverting the building blocks for fat, protein, and carbohydrate, and that these interconversions are under control of the intracellular equivalent of hormones. Then, by applying metabolic stress, such as that induced by raising the incubation temperature and so speeding metabolism, it was hoped that the need for intracellular hormones would outrun synthesis, and that these hormones or their precursors could be supplied exogenously. In essence, this is a crude attempt to study the 'adaptation energy' invoked by Selye (1959).

The initial experiments were on *Ochromonas malhamensis*, a choice dictated by its celebrated metazoan-like requirement for vitamin E_{12}, its phagotrophy, and considerations pointing to the primitiveness of chrysomonad animals. The early work showed that, as in thyroid-treated birds and mammals, the vitamin B_{12} requirement went up steeply with temperature; amino-acid antagonisms, and stimulations by purines, pyrimidines, and folic acid also appeared (Hutner *et al.* 1957). Enhanced metal requirements with heightened temperature were then recognized (Hutner *et al.* 1958a).

Poisoning by dinitrophenol, studied as a possible gauge of how

damage to oxidative phosphorylation figures in thermal injury or in the inhibition by various drugs being considered as alternative stress-inducing agents, proved different from thermal injury, as it was annulled by phosphate + glutamate (or compounds convertible to glutamate) (Hutner *et al.* 1958*b*).

We then decided to shift to *Ochromonas danica* (Pringsheim, 1955), a voracious, deep olive-green, exceptionally large, hardy chrysomonad, not requiring vitamin B_{12} (Heinrich, 1955). Identification of its 'temperature factors' has been speeded by the remarkable promotion of growth exerted by ketone-body acids (see Krebs, 1960). As this work is in progress, newer results will be outlined as they bear on the themes of this Introduction. We had long been disturbed by the seeming indispensability of carbohydrate for *Tetrahymena* (Holz, Erwin & Davis, 1959) and for *O. malhamensis*—a dietary restriction seemingly at variance with their omnivorousness, and which implied that the basal media for temperature experiments were unphysiological. The answers came from three directions:

(1) *Microbiological*: a polymer of β-hydroxybutyric acid was a food reserve of bacilli (Macrae & Wilkinson, 1958) and Athiorhodaceae (Stanier, Doudoroff, Kunisawa & Contopoulu, 1959); also *Ochromonas malhamensis* can utilize acetone (Vishniac & Reazin, 1958).

(2) *Metazoan*: as chief substrates, ruminants use fatty acids, notably acetic, propionic, butyric, and branched-chain acids, rather than carbohydrate (Annison & Lewis, 1959); also, many individual acids of the ketone-body cycle are readily oxidized by birds and mammals (see Krebs, 1960).

(3) *Enzymological*: β-hydroxybutyrate is readily oxidized by preparations of mitochondria from many sources.

Inclusion of β-hydroxybutyrate, butyrate, or amino acids yielding ketone bodies, in media containing acetate and a 'glycogenic' compound such as lactate or glycerol, allowed a full replacement of carbohydrate for *Tetrahymena* (D. Cox, personal communication) as well as *Ochromonas danica*; as expected, monobutyrin proved favourable, especially for *O. danica*. With media amply fortified with these compounds, the limit for *O. danica* could be raised from 36° to c. 39·2° and of *Tetrahymena pyriformis* var. 1 from c. 39·5° to 41·8°; high concentrations of amino acids, e.g. glycine, serine, leucine, isoleucine, and valine and methionine were required—a suggestive parallel, we think, to the long-known intense catabolism of protein and fatty acid in mammalian stress (Wilson, Moore & Jepson, 1958; Cuthbertson, 1960).

Apparently, as the temperature is raised, one mode of substrate utilization after another comes into play, some to capacity, until, at the

present upper temperature limit, an exaggerated catabolism of amino acids seems to be the source of the extra energy for growth (Frank *et al.* 1960). Dissimilation of propionic acid (a vitamin B_{12}-requiring reaction —Smith & Monty, 1959) coming in quantity from dissimilation of certain amino acids—e.g. valine, isoleucine, and methionine (see Greenberg, 1954), would account for the heightened B_{12} requirement seen in thyroid-treated higher animals and in *Ochromonas malhamensis* grown at elevated temperatures (Hutner *et al.* 1957).

Not unexpectedly, complex interactions of B vitamins, amino acids and substrates have been encountered and, at the upper limit, lipid requirements.

A comparison with the factors restoring the growth of rats treated with thyro-active materials might be instructive. Increasing the casein (Overby, Fredrickson & Frost, 1959) or lipids in the rations (Dryden, Riedel & Hartman, 1960) increased growth, but the active components in these and other supplements have not been identified. As the liver temperature of a normal rat exposed to 3° may rise abruptly 1° (Stoner, 1960), cold-stress may provide leads; nutritional information on cold-exposed rats seems wholly lacking.

Whether our results with *Ochromonas danica* mean anything for metazoa is problematical; they of course point to the molecular events that underlie environmental temperature barriers; as yet the only detailed information along these lines comes from an analysis of 'temperature' mutants. We have not yet sought seriously to push the temperature limits higher by feeding live food or using temperature-adapted inocula.

Oxyrrhis marine, a colourless marine dinoflagellate, seems to flout the idea that aerobic phagotrophs readily interconvert exogenous substrates: although able to feed on intact yeast cells, in pure culture no good substitute for acetate was found: many carbohydrates, Krebs-cycle acids, and butyric and propionic acids, also whole milk, egg yolk, and plant oils (Droop, 1959). This adds to the interest in working out the substrates for *Peranema*, which should reflect the characteristically 'acetate flagellate' habits of some of their *Euglena–Astasia* relatives.

INTERPRETATIONS AND A PROPHECY

What's the use of trying to educate ourselves?—we'll never know the ultimate secrets of life anyhow.
True, true—but let us joyously confirm our eternal ignorance through a million suggestive and stimulating contacts.
DON MARQUIS: *The Almost Perfect State* (1927).

Photosynthesis, sex, and animal origins

With protozoa so diversely made and variously adept, ancestor-hunting seems more of a needle-in-a-haystack enterprise. The hunt widens as, unexpectedly, the electron microscope confers patents of animality: the malaria organism is a phagotroph (Rudzinska & Trager, 1960), and *Trypanosoma mega* has a proper mouth (Steinert & Novikoff, 1960). The choice of experimental protozoa is narrowed by the desirability of the organism not being so highly adapted to life in nutritional deserts— fresh water or marine—that it copes poorly with culture media fortified for rapid growth, nor rendered so fastidious by the lush pickings of intracellular life as to demand almost prohibitively unstable enzymic cofactors. Provenance, however, is not much of a guide: *Ochromonas malhamensis*, for instance, which flourishes at American room temperatures in rich broths, came from cold, nutrient-poor Malham Tarn in Yorkshire (Pringsheim, 1952).

Resourcefulness is increasing in analysing the requirements of fastidious protozoa. Thus the carnivorous ciliate *Euplotes patella* can be reared on *Tetrahymena* if a guanine-antagonist such as 8-azaguanine is supplied (Lilly & Henry, 1956). (One wonders whether a high-ciliate diet would induce gout in a pig—pigs are susceptible to guanine gout: Bendich, 1955).

With the elucidation of most basic metabolic pathways, we can strive to discern common features in the responses of man and microbe to environmental change, e.g. it is now operationally useful to approach the eye-spot + flagellum + interposed transducer as 'the most elementary nervous system' (Wolken, 1956).

Desire to discern the connexions of environment, behaviour, and growth grows as old preoccupations lose urgency. The evolutionary success and venerability of voracious photosynthesizers—and laboratory domestication of some of them—makes pointless worry about the propriety of animals having photosynthesis; the problem in such protozoa is, in the formulation of Myers & Graham (1956), knowing how photosynthesis stretches time between bites. The ease with which *Euglena gracilis* can be 'cured' of its chloroplasts with streptomycin, or