The Leeuwenhoek Lecture, 1963

The size of small organisms

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[Plates 17 and 18]

Introduction

Leeuwenhoek's letters are good reading. They show us a man who was busily inquisitive about everything, with firm opinions and an emphatic way of expressing them. When he found that Hooke had printed part of one of his letters in a book, he commented pointedly that people had asked him to write a book himself but that he preferred not to (**2**, 361).* When told that people in Paris could not see the globules he described in many materials, he said he was absolutely unconcerned and advised them to come to Holland and be shown (**1**, 279). He saw the comic aspects of some of his work; the editors explain that in Dutch his reference to a recent letter on lice as 'my last lousy observations' has the same connotations as in English (**2**, 195). Nevertheless, he was conditioned by his time. He called in two Lutheran pastors to attest the truth of his observations (**2**, 257) and was at pains to emphasize that his studies on human sperm were made 'zonder eenige zondige bezoedeling van mij zelf'. Of more direct relevance to the theme of this lecture is his mechanical approach to the interpretation of nature. Repeatedly he tries to explain the pungency of pepper or ginger by postulating pointed particles in them that pricked the tongue, and he suggested that in purgatives the spikes are long enough to penetrate the layer of mucus on the gut wall. He assumed that these particles were too small to be resolved by his microscopes and so was delighted when he saw (**2**, 243) what he took to be narrow rods in eel blood; for he attributed to these the blood's ability to irritate the eye. He clearly postulated a micro-anatomy in what he saw and attributed physiological significance to it. It is fortunate that he was not a well-read and educated man. Had he been so he would have known the prolix confusion of Athanasius Kircher's microscopical observations and deductions and might have become a victim of what Charles Singer aptly calls the 'vermicular obsession' that, by the end of the seventeenth century, made people see worms in most diseased and many healthy organisms. Leeuwenhoek did not know what he was supposed to be seeing and was content to record what he saw.

Leeuwenhoek was interested in both aspects of our problem: that is to say, he wrote about the distribution and sizes of organisms, on the one hand, and about their origins, on the other. His interest in the small forms that he called 'diercken'

* All references given in this simple form are to *The collected letters of Antoni van Leeuwenhoek* published at intervals by Swets & Zeitlinger Ltd., Amsterdam.

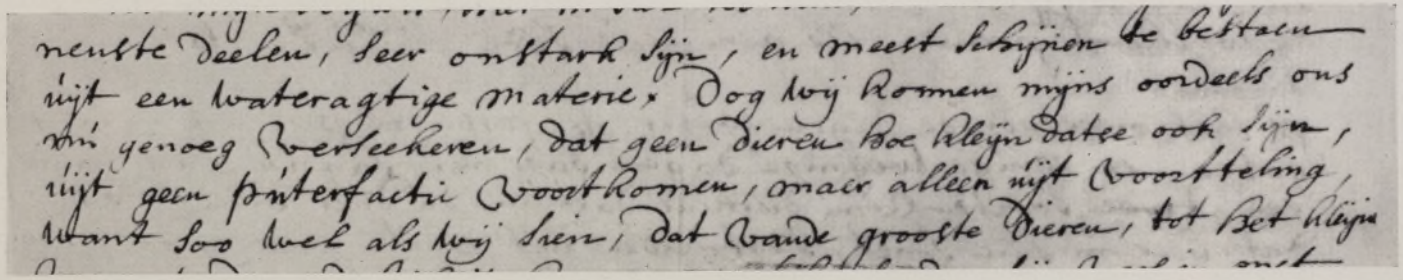
is well known. The word is generally translated as 'little animals' in English, but that seems to lose some of the intimacy of the original; 'beasties' would be a more suitable word. Leeuwenhoek discussed the origins of individual organisms, of species, and of organisms as a whole—that is, the origins of life. His criterion for deciding whether something was alive was straightforward; it was alive if it moved. Robert Brown become the eponym of the already well-known movement of small particles suspended in water, because of his convincing demonstration, in papers written in 1828 and 1829, that the movement was not a consequence of the animation of the particles. Movement as a criterion of life is, however, firmly embedded in language. It appears in such phrases as 'the quick and the dead' and in the opposition between oviparous and viviparous. The latter distinction has a special relevance because of Leeuwenhoek's strenuous opposition to Harvey's view that the foetus developed from an egg; it was obvious to him that fertilization depended on sperm, which could be seen wriggling, and that the mother was a mere incubator. This opinion led him to discuss the origin of species for he had to explain the appearance of maternal traits in the offspring of a cross. He decided it was a matter of nutrition; a she ass does not produce enough material to make the quantity of hair characteristic of a horse, and so produces a mule; babies with black mothers and white fathers are brown because they were supplied with what would now be called pigment precursors. He does not seem to have thought about the reciprocal crosses but he speculated extensively (**5**, 253) on the production of new species of fruit trees by altering the nutrition of the developing embryo.

Throughout the Middle Ages and Renaissance the origin of the simpler organisms did not seem to present any particular difficulties. The life histories of these organisms were unknown and it was easy to assume that they arose whenever conditions were favourable. By the seventeenth and early eighteenth centuries a few scientists, Harvey and Boyle, for example, were becoming sceptical. Leeuwenhoek was more forthright in disbelief (**3**, 79; **3**, 329) and argued that all the living forms he saw had developed from pre-existing forms. (See figure 2, plate 18 for the original letter and the Dutch transcript and English translation.) He discusses the transport of small organisms in air currents and describes (**6**, 63) an experiment with pieces of veal sealed in glass tubes that is very similar to the later and more widely known experiments of Redi, Spallanzani and Pasteur.

In the course of his work on micro-organisms, Leeuwenhoek, using a very simple microscope, discovered members of most of the groups that we now study. The only major group, interesting in the present context, that he missed, was the viruses; but this is hardly surprising because the criterion still generally used to decide whether an infective agent is a virus or not, is that it should be invisible even with a modern microscope. Furthermore, for reasons that I will enlarge on later, it is probably unwise to include viruses among the organisms; to do so would rob an otherwise useful category of its homogeneity. This ready recognition of organisms raises a question of fundamental interest and importance: why are they all so big? The question can conveniently be broken down into two subsidiary questions. What is the size of the smallest organism that can multiply independently of other organisms in a medium that has no metabolic activity of its own? How certain are we

FIGURE 1

(*Facing p.* 150)

a 

b ...binnenste deelen, seer onstark sijn, en meest schijnen te bestaen uijt een wateragtige materie. Dog wij konnen mijns oordeels ons nu genoeg verseekeren, dat geen dieren hoe kleijn datse ook sijn, uijt geen puterfactie voortkomen, maer alleen uijt voortteling, want soo wel als wij sien, dat vande grooste dieren, ...

c ... But in my opinion we can now be assured sufficiently that no animals, however small they may be, take their origin in putrefaction, but exclusively in procreation.

FIGURE 2. *a*, Extract from a page of Leeuwenhoek's holograph letter to Robert Hooke dated 12 November 1680. (Royal Society, Early Letters L 162).

b, *c*, Dutch transcription and English translation of the passage in *a* from the Collected Letters of Antoni van Leeuwenhoek, **3**, 328, 329.

entitled to be that we are not missing still smaller independent organisms because they do not obtrude themselves as pathogens? Little effort has so far been put into the deliberate search for metabolism in apparently sterile media. The time is now ripe to discuss whether biochemistry has reached a stage of development that enables a minimum size and complexity for an independent organism to be laid down.

The smallest free-living organisms

The simile used by Leeuwenhoek to describe the appearance of blood corpuscles, that they looked like sand grains on black taffeta, led Dobell (1932) to deduce that he used some form of dark ground illumination and so, with a magnification of only 200 to 300 diameters, was able to see objects only 1 μm* across. Most bacteria are larger than that but a few, such as *Brucella melitensis*, have only a third of that width though they are often elongated. The smallest cocci are 0·5 μm in diameter. The volume of these cells is therefore about 0·1 μm^3. This is the limiting size for organisms generally grouped among the bacteria though some can assume smaller *L*-forms. These are extremely interesting for they seem to be able to bring about the normal energy-exchange reactions but to have poor synthetic powers (Weibull & Beckman 1960). Still smaller organisms able to grow in inert media are grouped together as the Pleuropneumonia-like-organisms (*PPLO*) or Mycoplasmataceae. The pathogenic members of the group have, naturally, been most intensively studied but Laidlaw & Elford (1936) cultivated members of a similar group of organisms from sewage. A. Pirie (1937) found that one sewage organism could oxidize an extensive range of substrates *in vitro* so long as blood or haemin was present in the medium and she suggested that it was unable to conserve the necessary catalysts but was dependent on the medium for a supply. Although there is still no positive evidence that this is the explanation of the poor growth of *PPLO*, and of the complexity of the media that they need, the suggestion seems very reasonable. A similar state of affairs is now well known with the larger viruses. The rickettsiae lose ribonucleic acid and coenzymes, and with this loss become non-infective, but they recover infectivity when the coenzymes are restored (Moulder 1962). This leakage is probably an important factor forcing certain surface properties on organisms that do not adopt a wholly parasitic way of life. Though they may be able to make coenzymes they cannot survive unless they can retain them. As Dixon (1949) remarked, speaking of more highly organized tissues, the loss of coenzymes 'is what is really meant by the death of the tissue'.

PPLO multiply in inert media but they are unusually exacting in their nutritional needs, they grow slowly and attain only a small population density at the end (Butler & Knight 1960). There is disagreement whether these phenomena are a consequence of inadequacies in the medium, the presence of toxic components or products of *PPLO* metabolism, or of an intrinsic enzymic inadequacy. If leakage of coenzymes were the paramount defect, autocatalytic growth might be expected to start when this leakage had raised the concentration of coenzyme in the medium; but this is not observed. *PPLO* do, however, go through an interesting growth cycle (Klieneberger-Nobel 1962) in which the minimum reproductive unit, 125 nm

* μm = micrometre or micron (μ), nm = nanometre or millimicron (mμ).

across, seems to change from an electron-dense, sharply defined, granule into a larger ill-defined and diffuse mass. Within this a new generation of dense granules forms and the mass breaks up. It is clear from the electronmicrograms that the organism is pleomorphic, the sequence of events is not however unequivocal and there is evidence that the units are sticky and have a tendency to clump together (Morowitz *et al.* 1962). Whatever the sequence, it is tempting to think that synthesis is most active in the aggregated phase, that this may be because there is co-operation between the minimal units, and that this is made necessary by the absence of adequately impermeable cell walls.

No organisms as small as *PPLO* have been found in any other group. Thus the width of the smallest alga, *Micromonas pusilla* (Manton & Parke 1960), is about 1 μm. This is a very interesting organism, for it has reduced the normal cellular components to the numerical minimum, with a nucleus, plastid, Golgi body, mitochondrion and flagellum; but it has only one of each.

Clearly, other small organisms may exist that have not yet been found, either because of technical difficulties, or because they have not been looked for. Laidlaw & Elford deliberately sought organisms of less than bacterial size in sewage; the other members of the *PPLO* group were found because they cause a disease in man or a domestic animal. The viruses, though not strictly relevant to this discussion, serve to illustrate the point about difficulty of discovery. Without the criterion of disease-causation it would be difficult to recognize the presence of a virus. It would be carried in the host without symptoms and would have no metabolic activity. Only a very painstaking search for differences in the macromolecular constituents of many different putative hosts, of the same species, would reveal it. Similarly, the search for small organisms in an environment such as the sea or soil would involve looking for some change initiated in inert media by inoculation. This would be feasible by electron micrography or by studying changes in chemical composition, but the search would be very tedious. If there are organisms with virus-like dimensions, which might be called nanobionts, in the sea or soil they are probably largely adsorbed on the surfaces of mineral particles, detritus, or other organisms, and they may well be adapted to existence in two rather than three dimensions. This will complicate the search for them as it complicates the enumeration of other microbiota. A further complication arises from the widespread occurrence of antibacterial, antiviral, and antifungal substances in extracts from marine bacteria, algae and metazoa (Starr *et al.* 1962; Buck, Meyers & Kamp 1962). Some preliminary purification of the possible nanobiont to free it from inhibitory substances may be necessary before it can multiply.

Although the rarity of free-living organisms with diameters as small as 125 nm, and the absence of organisms smaller than that, may be spurious and may reflect only the difficulty or superficiality of the search, this size must for the time being be taken as a limit. It is worth while considering whether any satisfactory explanation for it can be found. The first step is to consider how many parts an independent cell must have and how big each is. Leeuwenhoek attempted this on several occasions. He saw (**3**, 297) structures inside erythrocytes that he interpreted as showing a subdivision into six units, and he went on to postulate that each of

these was itself made of six units. Prudently he stopped there although he had a great liking for sixfold subdivision and 1/36 is a fraction he repeatedly used. His idea that biology was inextricably bound up with the number six was a harmless eccentricity, but Swedenborg (1740) took it very seriously. Anyone who wades through all his pages of hexamania, and eulogies on Leeuwenhoek, will tend to agree with Blake (1793) 'Any man of mechanical talents may, from the writings of Paracelsus or Jacob Behmen, produce ten thousand volumes of equal value with Swedenborg's, and from those of Dante or Shakespear an infinite number.' Many microscopists since have tried to interpret structures at the limit of resolution and some electronmicrographers have succumbed to the same temptation.

Early estimates of molecular dimensions

When Leeuwenhoek considered the internal structure of the organisms that he studied, he thought of them as miniature versions of more familiar organisms. Sometimes he states this explicity (**2**, 391).

'But many of our imaginations and investigations of nature are futile, especially when we see little living animals and see their legs and must judge the same to be ten thousand times thinner than a hair of my beard, and when I see animals living that are more than a hundred times smaller and am unable to observe any legs at all, I still conclude from their structure and the movements of their bodies that they do have legs (and therefore legs in proportion to their bodies, just as is the case with the larger animals upon which I can see legs). Taking this number to be about a hundred times smaller, we therefore find a million legs, all these together being as thick as a hair from my beard, and these legs, besides having the instruments for movement, must be provided with vessels to carry food.'

The idea comes in elsewhere by implication; thus he postulates (**3**, 397) skin, veins, nerves and muscles, and, for the last, proposes a 'Chinese box' construction with fibres inside fibres inside fibres (**3**, 397). But he realized that there must be a limit somewhere because he believed in the existence of atoms. On the basis of his assumptions about internal structure he concluded (**3**, 59) that the ultimate particles of water must be able to get through capillaries that had 6×10^{-12} the diameter of a grain of sand but later (**5**, 27) proposed a more realistic size. Descartes suggested that water particles were eel-like; Leeuwenhoek argues that they are globular and fill space because they are plastic and pack together as bladders full of water do in a barrel; this leads him to suggest that their volume is 10^{-18} that of a grain of sand. If sand grains are taken as 0·86 mm in diameter that would give water molecules a diameter of 0·86 nm and this is very interesting as an early attempt to estimate molecular dimensions. This estimate, made in 1685, is rather nearer actuality than Halley's (1691) based on the thickness of the thinnest coherent gold film on gilded silver wire. He calculated that this was 1/134500 of an inch which, if the film was 1 atom thick, leads to 0·2 μm for the diameter of an atom of gold.

This excursion, by a biologist, into molecular theory, justifies a digression on the part played by biological lines of thought in early attempts to establish molecular sizes. So far as I know the first measurement was made by Magnenus (1646) who

meditated on the complexity that it was necessary to assume in the head or foot of a midge and went on to observe the amount of incense needed to produce a perceptible smell in a room. He found that the volume of the room was 777 600 000 times that of the grain of incense. For obscurely phrased reasons he argued that one thousandth of a grain would have been sufficient and that each scent particle probably contained a million elemental atoms. Hence there are $7{\cdot}8 \times 10^{17}$ atoms in a piece of incense as large as a chick-pea. Whatever the reasons behind his assumptions, it is interesting that his value is only out by a factor of about 1000. Erasmus Darwin made no measurements but he had strong preconceptions and realized that the preformationist picture of embryonic development was incompatible with the existence of atoms of a size that seemed to him reasonable. His wording (1801) is interesting '...that these infinitely minute forms are only evolved or distended, as the embryon increases in the womb. This idea, besides its being unsupported by any analogy we are acquainted with, ascribes a greater tenuity to organized matter, than we can readily admit; as these included embryos are supposed each of them to consist of the various and complicate parts of animal bodies: they must possess a much greater degree of minuteness than that which was ascribed to the devils that tempted St Anthony; of whom 20 000 were said to have been able to dance a saraband on the point of the finest needle without incommoding each other.' His grandson was less convinced that matter was not infinitely subdivisible. He records that he had read the *Zoonomia* 'but without producing any effect' and this is clearly shown in the theory of pangenesis by means of gemmules which reproduced themselves, were susceptible of modification which they then transmitted to subsequent gemmules, and pervaded all parts of an organism. Darwin (1868) refers to this as a 'provisional hypothesis' but it is set out in an unexpectedly declamatory and emotional way with a rather casual use of words such as *infinite* and *inconceivable*. Nevertheless, it is interesting that at only one point in the chapter does he seem troubled by the complexity of the inheritance-mechanism postulated. To quote: 'Excessively minute and numerous as they are believed to be, an infinite number derived, during a long course of modification and descent, from each cell of each progenitor, could not be supported or nourished by the organism'. But Charles, unlike Erasmus, did not realize that there simply would not be room for all the gemmules he was postulating.

The difficulty was clearly recognized by Galton (1872) who said succinctly 'The heritage of peculiarities through the contributions of 1000 consecutive generations, even supposing a great deal of ancestral intermarriage, must far exceed what could be packed into a single ovum', and it was satisfactorily resolved by the suggestion that only a representative group of traits was transmitted. Galton does not seem to have known that Loschmidt (1865), using the dynamical theory of gases and his measurements of their viscosity, had already determined some molecular magnitudes and that these values had been accepted by Kelvin, Clerk Maxwell, and others so that the matter could be put on a firm quantitative basis. But Maxwell (1875), whose physiological interests are well known, argued that there was only room in the smallest organisms, or in a gamete, for a million molecules of average size and that, if these molecules had no individual properties other than position

and velocity, there was a limit to the amount of complexity that could be transmitted. He gently ridiculed Galton's ideas but all he was attacking was a certain looseness of phrasing, and he fell into the same error himself in speaking of the infinite complexity of an organism; Galton had been trying to envisage a mechanism for ensuring heredity within the limits set by the dimensions of a cell. The problem was thus clearly recognized and all that should have been called for was more observation and measurement, but speculation and notional biochemistry continued. Darwin's gemmules got companionship from Weismann's biophors, Tos's bionomes and so on. But we should not be too hard on the biologists; it was difficult to convince chemists at that time that Democritus and Dalton were right, that atoms had a real existence and determinable size, and that the observed regularities of chemistry depended on more than the preferred combining ratios of structureless stuff. Ostwald remained unconvinced until 1909 and conviction then came from Perrin's (1909) paper 'Mouvement brownien et réalité moleculaire'. It is interesting that even at that date Perrin thought it necessary to put the word *réalité* in the title. As acceptance of molecularity became general, attempts were made (e.g. MacKendrick 1901) to calculate the number of molecules in a cell; these were frustated because the sizes of colloidal molecules were not then known. A sketch of the development of our ideas about the sizes of enzymes and other biologically active macromolecules has been given elsewhere (Pirie 1962*a*). We now seem to have the essential quantities needed to answer the question that Leeuwenhoek posed. Qualitatively however many problems remain.

Criteria for an organism

The basic qualitative problem is to distinguish an analogy from an organism. At a time when it was difficult to initiate fire, Heraclitus compared life to fire; the comparison with the reproduction of magnets is closer; and Pasteur compared it to crystallization. The last comparison is particularly interesting because the first experiments in which bacteria were effectively filtered out of air were undertaken by Schroeder (1859) who was trying to find out the minimum size that was needed for a crystal to be an effective 'seed' for a supersaturated solution. There is a widespread illusion that crystallization is not a close analogy because the substance that crystallizes pre-exists in the supersaturated solution. It often does. But one would have to abandon the ionic hypothesis if one insisted that this is always so; it is the component ions of ferrous ammonium sulphate, rather than the complete structure, that pre-exist. The essence of these analogies is that a system should remain unchanged until a small addition is made to it and that it should then produce abundant copies of whatever is added. The growth of chemical knowledge has produced many examples of this phenomenon. The conversion of trypsinogen into trypsin by the addition of a trace of trypsin is well known and so is the initiation of polysaccharide formation when a seed of polysaccharide is added to a mixture of phosphorylase and hexose phosphate (Cori & Cori 1939; Hanes 1940). Calvin (1960) points out the relevance of even simpler autocatalytic systems such as the reduction of cupric ions to cuprous by molecular hydrogen; there is a prolonged lag unless a trace of cuprous ion is added.

In practice, decision about the biological origin of a structure is often reached on purely aesthetic grounds. We are familiar with biological form and regard it as excessively unlikely that certain structures could have arisen in any non-vital way. It is for this reason that the most probable time for the origins of life now seems to be about -2 Gyears; it seems capricious to try to interpret structures found in some ancient rocks in any non-biological way (cf. Cloud & Abelson 1961). The similar attempt to assign a vital origin to certain structures in meteorites is not generally accepted and all the structures described so far have been plausibly interpreted as the result of crystallization or recent contamination.

Reliance on aesthetic criteria for recognizing organisms is valid only after full account has been taken of the readiness with which certain systems integrate themselves without vital intervention. Crystallization patterns and the discontinuous precipitations studied by Liesegang are obvious examples; the readiness with which collagen fibres reform from dispersed collagen (Randall *et al.* 1955) and tobacco mosaic virus rods from disintegrated virus should also be borne in mind. Fox & Yayama (1963) find regular particles, that take the Gram stain, in the products of thermal polymerization of amino acids. Clearly, organisms can be recognized unequivocally only when a very elaborate structure, or a metabolic activity, is found.

At this stage in discussions about the nature and origins of life it is customary to consider viruses; they played a prominent part in the international symposium on the origins of life held in 1957 (Oparin *et al.* 1959). Viruses are a rather more far-fetched analogy than those already mentioned, because viruses depend on the well-integrated catalytic systems of a host cell. This dependence is complete; most viruses seem to lack all enyzmes though some contain enzymes concerned with the process of invading a cell rather than with multiplication within it. Conventional obligate parasites, on the other hand, contain an array of enzymes but have to rely on the host for a few of them, or for their products. There is no reason to think that the smaller viruses have any action in their hosts other than misdirecting the synthetic mechanisms.

In the limit the multiplication of a virus could be strictly comparable to crystallization (Pirie 1937) with the invader acting as a focus on to which host components, normally present in very small amount, aggregate. This extends to viruses the idea, at least as old as Kant, that growth in a biological system has points of resemblance to growth and repair in crystals. But it is more probable that infection with small viruses is a derangement of the nucleoprotein metabolism of the host (Pirie 1950*a*; Bawden & Pirie 1953). That is to say, the pre-existing pattern of synthesis is to some extent reorientated so that virus, rather than the normal cell substance, is made. There is no reason to assume that the components of the virus are entirely novel and that it must bring into the cell its own complete specification; trivial changes in the normal specifications could be sufficient to initiate the uncontrolled synthesis of an anomalous product that is characteristic of infection (Pirie 1958).

Unless these considerations are borne in mind, the virus analogy might well be extended to include any metabolic product that stimulates its own formation in an

organism. Thus various substances produce plant galls. It is reasonable to assume that one will be found that causes the formation of a gall that contains more of the initiating substance; and it might well, like an auxin, be readily synthesized (Pirie 1948*b*); similarly, Lederberg (1952) described an occasion on which the OH ion nearly qualified as an infective agent. Viruses are admirable tools for the study of many aspects of the behaviour of organisms but, because of their dependence on a host, only confusion results if they are regarded either as organisms or as stages in their evolution (Pirie 1963). It is, nevertheless, an interesting fact that virus-like behaviour has not so far been found in any material free from macromolecular nucleic acid.

The components of an organism

Having disposed of analogues, and recognized that the apparent limit to the smallness of free-living organisms may be technical rather than real, we can discuss the light that is thrown on the problem by our knowledge of the structure of enzymes and the integration of their reactions. Even if organisms do not of necessity have some minimum size, the enormous number of forms with volumes greater than a few μm^3 suggests that a size of that order is very advantageous. There is no known example of an organism, regardless of its size, that exists by virtue of only one catalytic process, and if such a system were found it would probably be related with crystallization, or the change from metallic to grey tin, rather than with anything biological. We do not know what the minimum number of enzymes, or catalysts, is; Haldane (1957) suggested four, Hunter (1963) estimates that there are 1000 enzymes in *Escherichia coli*. Whatever the number, the fundamental problem is to get them so disposed that they can act in an integrated manner. John Hunter seems to have realized this, for notes (Palmer 1835) taken during his lectures state that '. . . matter is so arranged that the principle of life arises out of the arrangement. . . '; but few will now agree with the further idea that the principle was something, like magnetism, 'superadded' to matter.

Effective catalysis depends on enzymes and they, without exception, have molecular weights in the thousands. Langenbeck (1949) and Calvin (1960) give examples of the dramatic increase in catalytic efficiency in a series of enzyme models of increasing size, culminating in the enzyme itself. There is no satisfactory explanation for this, nor for the fact that all enzymes so far purified use the architecture of a protein to get the apparently necessary size. It is possible (Pirie 1959) that size is important because, simply as a result of inertia, it stills Brownian movement; it is also possible that size is important because it allows one molecule partly to encompass another so that contact, sufficiently prolonged for chemical changes to take place, becomes possible without the necessity for the covalent type of bonding that is necessary with small molecules. The general use of proteins to fulfil these objectives may be an evolutionary accident. The fact that all organisms seem to use them may show no more than that they were an early and reasonably efficient system of catalysis. Once any system is thoroughly established, organisms using it would, as Darwin and many others towards the end

of the last century realized, tend to eliminate the preliminary, and presumably inefficient, first steps towards any other catalytic system even although the rival system, had it been given time to develop, could have proved as, or more, efficient. Tenancy of a metabolic niche offers great advantages.

The basic problem of contemporary biochemistry is not how a reaction is carried out, nor how its specificity is controlled, but how energy derived from one process is used to drive another. For many years Szent-Gyorgyi (e.g. 1960) has been trying to wean biochemists from a mechanical approach to energy transmission; he has argued that proteins can act as semiconductors leading electrons from one site to another and that energy can move, delocalized, as a wave. He invokes such a process in the initial energy adsorption phase of photosynthesis (Avery, Bay & Szent-Gyorgyi 1961).

There is general agreement that photochemical activity disappears at a certain stage in the disruption of chloroplast fragments and it is claimed (Milner, Koenig & Lawrence 1950) that activity is restored by re-aggregation. The mechanism is not known but it would repay study as a means of getting evidence about the factors underlying the large size of the minimal photosynthetic unit. Various complexes, e.g. those involved in metachromatic staining, can be formed with macromolecular substances only. Electric conduction is also suggested, in a stimulating speculation by Marshak (1958), as an agent in many aspects of the economy of the cell; he suggests that the various helical structures seen in cells may produce biologically important magnetic fields, and that the regular arrays that are now often seen in electronmicrograms may be absorbing electromagnetic radiation. Newton (1712) may have been showing his usual prescience when he remarked in Proposition 12 of the 'Scholium generale' *Nutritionem per attractionem electricam peragi*.

Whatever the reason, organisms seem to depend on a minimum number of enzymes each with a minimum size: this establishes a minimum weight for the organism that is somewhere between 1 and 30 Md.* This is so much smaller than the observed minimum weight that it is necessary to look for some entirely different restriction on size. The most obvious is structural organization and this leads immediately to one of the most general problems in biology. Organisms from scattered parts of the biological hierarchy often use similar structures or processes. Is this because these organisms have a common ancestry, or because there is no other equally effective way of achieving the result? As examples the use of *ATP*, of carotenoids in vision, and the 9 + 2 fibre structure of flagella may be mentioned. The aspect of this uniformity that is relevant in this discussion is the striking similarity found among cell walls, mitochondria, microsomes and other subcellular components. If this uniformity arises because the organisms that have been studied have a common origin and the proto-organism used a certain structure, little can be argued from the uniformity—it could simply be an accident that is perpetuated because the possible alternative systems have so far been associated with organisms that were, in other ways, less efficient than those derived from the proto-organism. But if the uniformity arises of necessity because there is no other

* Md = dalton $\times 10^6$. (The dalton is $\frac{1}{12}$ of the mass of an atom of ^{12}C.)

substanceor structure that can work with an efficiency comparable with that of the one with which we are familiar, the uniformity becomes significant for the present discussion. This state of affairs is familiar to students of human artifacts. Use of the same symbol or decorative design by two communities is *prima facie* evidence for contact; use of the wheel is not because there is no alternative to it. It will be assumed in what follows that the uniformities in biological structure come in of necessity.

When catalysts act in sequence bringing about progressive changes in a substrate, or adding successive pieces during the synthesis of a macromolecule, they must be arranged in an orderly manner. The matrix could be a thread, net, or sponge. If it had one of these forms, transfer of metabolites from catalyst to catalyst would have to be efficiently managed or the intermediate stages would be lost by diffusion. Any system in which the catalysts are enclosed in a bag of suitable permeability would have obvious advantages, and this form of enclosure would, as has already been pointed out, help to conserve the coenzymes on which so many enzymic processes depend. The conclusion seems inescapable that a free-living organism must be enclosed so that the environment in which its catalytic systems work differs from the outside environment that supplies substrates and receives metabolic by-products. In other words, a cell wall or membrane is an essential minimum requirement.

In a discussion of minimal requirements, the bacterial cell wall is not relevant; two symposia of the Society for General Microbiology have shown (Miles & Pirie 1949; Spooner & Stocker 1956) the multiplicity of functions that it performs. What we are concerned with is the thickness of the thinnest biological membrane that has physical coherence. There is an interesting uniformity about such membranes regardless of their origin. Thus the outer membranes of protozoa (Pitelka 1963) and blue-green algae (Echlin 1963), and the plasma membrane of bacteria and funguses (Robinow 1962) are about 8 nm thick. This is also the thickness of the membranes surrounding mitochondria and the nucleus, and making the Golgi lamellae in some tissues from vertebrates (Yamamoto 1963). Rabbit mitochondria (Glauert *et al.* 1963) seem to have a slightly thinner membrane. All these measurements are subject to the usual uncertainties of electronmicrography because the sections measured may not always have been cut normal to the surface of the structure being sectioned, while positive staining tends to exaggerate and negative staining to minimize the width of an object. Nevertheless, the uniformity is impressive and so is the three-layered appearance of the membrane; this is generally interpreted as two protein layers separated by a layer of lipid. There is no reason to regard these membranes as inert mechanical containers. During the past 2 years a regular hexagonal pattern has been seen in several of them in many different laboratories. The possibility has been mooted that this is an artifact because a similar pattern appears when cholesterol films are treated with saponin, and saponin and similar polar substances were used in making some of the preparations. This in some ways increases the interest of the structures. It shows that processes independent of organized metabolism can lead automatically to structures similar to those seen in biological systems. More refined techniques (Blair *et al.* 1963) suggest that the

mitochondrial membrane is covered with a hexagonal array of knobs and that these are enzymically active. Houwink (1953) found a similar array in bacteria and Murray (1963) gives an interpretation of its detailed structure. It may be worth bearing in mind the possibility that the resemblance of these structures to miniature versions of the *T*-even phages is more than a coincidence; if there is any substance to the suggestion (Pirie 1962*b*) that a virus is the 'caricature 'of some normal cell component, these knobs would be strong candidates for the role of the normal model that is being caricatured.

Bacteriophages are relevant for another reason; as a result of osmotic shock and some other treatments they give a protein coat, or 'ghost', that is stable enough to be handled in solution. These membranes have not been studied in the same detail as those already discussed, but the morphology of the virus, the ratio of coat weight to total weight and the appearance in electronmicrograms (Bacq & Horne 1963) suggest a thickness of the order of 3 nm. Similarly, both *in vitro* and *in vivo* turnip yellow mosaic virus produces hollow protein shells with a wet diameter of 31 nm. The shell makes up about $\frac{2}{3}$ of the total volume so its thickness is 5 nm. Huxley & Zubay (1960) state that the shell wall is 2 to 3 nm in dry preparations.

From these results it is clear that a stable membrane can be as thin as 5 nm but they do not show that this is the limit because the membranes that have been discussed are either metabolically active or fall into a special category through being part of a virus structure. At first sight cellulose seems to have potentialities as an inert material from which a thin membrane could be made, but cellulose sheets appear invariably to be made up of a series of lamellae which are themselves made of parallel fibres. The claim that cellulose microfibrils can be as thin as 3 nm is disputed (Colvin 1963) and the width originally given, 10 nm (Preston & Kuyper 1951), seems a reasonable minimum. To get stability, with this mode of construction, at least two lamellae seem necessary. The mucopeptides primarily responsible for the rigidity of bacterial cell walls seem likewise to make an open net structure through which particles as large as 0·1 Md can pass (Work 1961). They would not therefore, when unreinforced, serve to retain coenzymes.

Internal organization

It was obvious to early scientists that an organism had an intricate internal organization. Thus Jean Rey (1630), when discussing the increase in weight which occurs when lead is turned into the oxide, dismissed the idea that it could be a vital effect with the words 'Car touchant sa vie, comment en auroit le plomb, puis qu'il est un corps homogenée, sans distinction de parties, sans organes, & sans aucun effect ou action vitale ' It is odd that Hopkins had to put so much devoted effort into getting general acceptance for the same idea in this century; but it is accepted and no one now regards a cell as a bag full of enzymes moving freely in solution. The size of the organizational units is therefore highly relevant in discussing the minimum size of an organism. Important as the mitochondria are in the dynamics of cells in many different groups of organisms, including the single-celled algae (Manton & Parke 1960), they are not relevant here because structures that can be regarded as typical mitochondria are not found in bacteria; though particles are

present that seem to have similar activities; furthermore, most mitochondria are as large as the smaller free-living organisms.

Soon after agreement had been reached about the general properties of viruses, uninfected tissues were studied and ribonucleoprotein particles in the same size range were found in animals (Claude 1940, 1949) and plants (Pirie 1950*b*). They have been given various names, such as microsomes, ribosomes, ergosomes and lysosomes, but the distinctions are by no means sharp. As Graham (1861) remarked apropos the distinction he was making between crystalloids and colloids, '. . . maxim that in nature there are no abrupt transitions, and that distinctions of class are never absolute'. The particles from tobacco leaves are 20 to 30 nm in diameter when dried for electronmicrography and have a strong tendency to adhere to one another linearly (Pirie 1950*b*), they are associated with several enzymes, including ribonuclease, and they are unstable *in vitro* though their stability is greater in the presence of magnesium ions (Holden & Pirie 1955; Pirie 1957*b*), which inhibit ribonuclease. Much recent work on the so-called soluble, and messenger, ribonucleic acid is vitiated by inadequate recognition of the extent to which ribosomes autolyse during isolation. Ribosomes made from other tissues are similar in many respects to those from tobacco, but there is no reason to assume that, even in one tissue, they are all alike. The converse is indeed probable, for it is generally wise to assume heterogeneity in a biological system until homogeneity has been demonstrated.

There is some conflict of opinion about the status of the enzymes associated with these particles—some may have been adsorbed during the isolation; there is also conflict over their state *in vivo*—whether they exist as separate units, small aggregates, or as nodes in the more elaborate structure of the ergastoplasm. But there is now general agreement that they are metabolically important structures in many, if not all, organisms. If they are indeed an essential feature of a free-living organism, it is obvious that the number that have to be packed into each is one of the quantities we should know if we are to discuss an organism's limiting size.

Bradfield (1956), on the basis of some reasonable assumptions, concluded that there was room in a microsome for only fourteen protein molecules weighing 35000 d and therefore that these particles, if they are the site of much of the metabolic activity of a cell, must be a heterogeneous group the members of which have specialized functions. In the presence of suitable substrates and energy sources, such as *ATP*, individual microsomes incorporate amino acids, but there is no definite evidence that they can synthesize and liberate proteins. Microsomal aggregates however can do this (Goodman & Rich 1963; Noll, Staehlin & Wettstein 1963). Protein synthesis is such a fundamental and fascinating theme that it is now getting a great deal of attention and generalization is hazardous, but there seems to be a considerable measure of agreement. Protein synthesis is pictured as taking place on each unit of a row of microsomes through which a ribonucleic acid filament is being drawn. The nucleic acid is thought to control the sequence in which the amino acids are added to the growing protein molecule in much the same way that the holes in the cards of a Jacquard loom control the pattern and those in a 'Pianola' roll control the tune. It would be unreasonable to take such a

mechanical picture altogether seriously, but it is a stimulating beginning and it suggests that each microsome may be able to make several different proteins, enzymes for example, under the influence of different instruction filaments.

Proteins now dominate our thinking about biology; they also appear to dominate biology itself because of their role as enzymes, although I have argued elsewhere (Pirie 1948*a*, 1957*a*) that this may be the result of selection for efficiency rather than being an essential attribute of an organism. For this reason the argument, so far, has been directed at protein synthesis; another reason for the direction is that protein synthesis has recently received a great deal of skilled experimental attention. It is now a text-book commonplace that sites of protein synthesis are rich in ribonucleic acid. The word protein is intrusive in that statement. Sites of synthesis, or indeed of metabolism in general, are rich in ribonucleic acid. It may well be therefore that many forms of synthesis proceed in a manner similar to protein synthesis though, as has been pointed out, some polysaccharides can be synthesized by a much simpler system once a model is available. The reason for this distinction is by no means obvious.

Conclusion

If we assume that the survival of an organism in the predator-filled contemporary environment depends on the integrated action of several dozen enzymes, that their synthesis depends on a microsomal mechanism similar to those so far investigated, and that the action of some of them depends on the retention of coenzymes by a relatively impermeable membrane, the fact that the smallest known free-living organisms are about 0·1 μm wide seems eminently reasonable. That gives about the volume that would be needed for a 5 nm cell wall and 20 to 30 microsomes. An organism as simple as this would be saprophytic and would need an environment that supplied an energy source and many of the amino acids and other metabolites. It may be that the rarity of nanobionts is simply a reflexion of the rarity of suitable natural environments supplying these things.

This need not always have been the situation. There is now general agreement (e.g. Oparin *et al.* 1959) that it is reasonable to postulate a probiotic environment rich in a varied group of organic molecules. Polymerization is such a standard feature of chemical reactions in the laboratory that there is no difficulty about postulating the non-vital formation of macromolecules. There is less agreement about the next stage in biopoesis. It is by no means obvious why it is so often assumed that the first catalytic systems, so fully integrated that they could be regarded as stages in the evolution of saprophytic organisms, should have been very small. Macromolecules are notoriously prone to flocculation and the floccules are as likely a site as any other for primitive catalysis. Similarly, there is no reason to assume that there was a steady accretion of capacity within one unit during the early phases of the origins of life. Bastian (1872) claimed that the organisms, of whose spontaneous generation he was such an enthusiastic advocate, were formed by the coalescence of smaller semi-vital particles; the somewhat analogous reconstitution of sponges after dispersal was extensively studied and discussed at the beginning of this century; and Haldane (1954) suggested that the

first system that could be properly called an organism arose through the accidental fitting together of several semi-vital units. This is an eminently reasonable suggestion and there is no need to specify the chemical nature of the original components, some may well have been catalytically active mineral surfaces (Pirie 1948*a*; Goldschmidt 1952) and these may have been extensive.

This discussion of minimal vital requirements has therefore no necessary connexion with the problem of the origins of life. The nanobionts may be derived secondarily by devolution. As often, the point may be made clearer by analogy—or allegory 'which decorates the house already built by argument'. Starting with the 'beast-machine' of Descartes or La Mettrie, and considering the stages by which its complexity can be diminished, we can proceed in many different ways, but there is only space to discuss the two that differ most radically. The mechanism of self-construction can be preserved while the ability to make the necessary components is sacrificed. This leads to the type of machine postulated by von Neumann which can assemble itself from a limited number of pre-existing parts. This would be analogous to an organism of the *PPLO* group in that it would contain the integrative mechanism but would be unusually exacting in its nutritional requirements. Simplification could, on the other hand, proceed by the elimination of the integrative mechanism leaving only the instructions that have to be followed by another machine to make, not a new machine, but a new copy of the instructions. That line would produce an analogy of the viruses which, as I have said, are irrelevant because their existence presupposes the continuing presence of a more elaborate machine or organism. Both modes of approach start with a fully independent system and whittle it away, and it is reasonable to postulate such a process as the mode of origin of viruses and *PPLO*. Obviously no similar process could have been involved in biopoesis because the probiotic earth could not, by definition, have contained a system ripe for whittling away. If this analysis is correct, the study of the smallest organisms is interesting from the practical standpoint of pathology, or as a means of understanding the essential nature, if any, of life, but it is not a means of studying life's origins.

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