Some Developments of Modern Zoology.[1]

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ZOOLOGY has far outgrown its early boundaries when it could be defined simply as a part of natural history, and at no period has its growth been more rapid or more productive in results of scientific and practical importance than during the last two or three decades. That period has witnessed a growth of our knowledge of the living organism of the same order of importance as the progress in our knowledge of the atom. Never have investigators probed so deeply or with so much insight into the fundamental problems of the living animal; the means for observation and recording have become more delicate, and technique of all kinds more perfect, so that we can perceive details of structure and follow manifestations of activity of the organism which escaped our predecessors.

Among the notable features of zoological activity during the last twenty-five years, the amount of work on the physiology of organisms other than mammals must attract early notice in any general survey of the period. Eighty years ago Johannes Müller's physiological work was largely from the comparative point of view, but for some years after his death the comparative method fell into disuse, and the science of physiology was concerned chiefly with the mode of action of the organs of man or of animals closely related to man, the results of which have been of outstanding importance from their bearing on medicine. Interest in the more general applications of physiology was revived by Claude Bernard ("Leçons sur les phénomènes de la vie," 1878), and the appearance of Max Verworn's "General Physiology," in 1894, was in no inconsiderable measure responsible for the rapid extension of physiolcgical methods of inquiry to the lower organisms—a development which has led to advances of fundamental importance. Many marine and freshwater organisms lend themselves more readily than the higher vertebrates to experimentation on the effects of alterations in the surrounding medium, on changes in metabolic activity, on the problems of fertilisation and early development, on the chemistry of growth and decline, and to the direct observation of the functioning of the individual organs and of the effects thereon of different kinds of stimuli. The study of these phenomena has greatly modified our interpretation of the responses of animals and has given a new impetus to the investigation of the biology and habits of animals, *i.e.* animal behaviour.

This line of work—represented in the past by notable contributions such as those by Darwin on earthworms, and by Lubbock on ants, bees, and wasps—has assumed during the last two or three decades a more intensive form, and has afforded a more adequate idea of the living organism as a working entity, and revealed the delicacy of balance which exists between structure, activity, and environment.

The penetrating light of modern investigation is being directed into the organism from its earliest stage. During the summer of 1897 Morgan discovered that the eggs of sea-urchins when placed in a 2 per cent. solution of sodium chloride in sea-water and then transferred to ordinary sea-water would undergo cleavage and give rise to larvæ, and J. Loeb's investigations in this field are familiar to all students of zoology. Artificial parthenogenesis is not restricted to the eggs of invertebrates, for Loeb and others have shown that the eggs of frogs may be made to develop by pricking them with a needle, and from such eggs frogs have been reared until they were fourteen months old. The application of the methods of micro-dissection to the eggs of sea-urchins is leading to a fuller knowledge of the constitution of the egg, of the method of penetration of the sperm, and of the nuclear and cytoplasmic phenomena accompanying maturation and fertilisation, and will no doubt be pursued with the object of arriving at a still closer analysis of the details of fertilisation.

The desire for more minute examination of developing embryos led to the more careful study of the egg-cleavage, so that in cases suitable for this method of investigation each blastomere and its products were followed throughout development, and thus the individual share of the blastomere in the cellular genesis of the various parts of the body was traced. This method had been introduced by Whitman in his thesis on Clepsine (1878), but it was not until after the classical papers of Boveri on Ascaris (1892) and E. B. Wilson on Nereis (1892) that it came into extensive use. For the next twelve or fifteen years, elaborate studies on cell-lineage formed a feature of zoological literature and afforded precise evidence on the mode of origin of the organs and tissues, especially of worms, molluscs, and ascidians. A further result of the intensive study of egg-cleavage has been to bring into prominence the distinction between soma-cells and germ-cells, which in some animals is recognisable at a very early stage, *e.g.* in Miastor at the eight-cell stage. The evidence from this and other animals exhibiting early segregation of germ-cells supports the view that there is a germ-path and a continuity of germ-cells, but the advocates of this view are constrained to admit there are many cases in which up to the present an indication of the early differentiation of the germ-cells has not been forthcoming on investigation, and that the principle cannot be held to be generally established.

A cognate line of progress which has issued from the intensive study of the egg and its development is experimental embryology—devoted to the experimental investigation of the physical and chemical conditions which underlie the transformation of the egg into embryo and adult. By altering first one and then another condition our knowledge of development has been greatly extended. By artificial separation of the blastomeres the power of adjustment and regulation during development has been investigated, and by further exploration of the nature of the egg the presence of substances foreshadowing the relative proportions and positions of future organs has been revealed in certain cases, the most striking of which is the egg of the Ascidian *Cynthia partita* (Conklin, 1905).

Progress in investigation of the egg has been

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paralleled by increase in our knowledge of the germ-cells, especially during their maturation into eggs and sperms, the utmost refinements of technique and observation having been brought to bear on these and on other cells. During the last thirty years, and especially during the latter half of this period, cytology has developed so rapidly that it has become one of the most important branches of modern biology. One of the landmarks in its progress was the appearance, at the end of 1896, of E. B. Wilson's book on "The Cell." A great stimulus to cytological work resulted from the rediscovery in 1900 of the principle of heredity published by Mendel in 1865, which showed that a relatively simple conception was sufficient to explain the method of inheritance in the examples chosen for his experiments, for in 1902 Sutton pointed out that an application of the facts then known as to the behaviour of the chromosomes would provide an explanation of the observed facts of Mendelian inheritance. In the same year McClung suggested that the accessory chromosome in the male germ-cells is a sex-determinant. These two papers may be taken as the starting-point of that vast series of researches which have gone far toward the elucidation of two of the great problems of biology—the structural basis of heredity and the nuclear mechanism correlated with sex. The evidence put forward by Morgan and his colleagues, resulting from their work on Drosophila, would seem to permit little possibility of doubt that factors or genes are carried in the chromosomes of the gametes, and that the behaviour of the chromosomes during maturation of the germ-cells and in fertilisation offers a valid explanation of the mode of inheritance of characters. The solution of this great riddle of biology has been arrived at through persistent observation and experiment and by critical analysis of the results from the point of view of the morphologist, the systematist, the cytologist, and the geneticist.

Among other important developments in the period, reference may be made to the great activity in investigating the finer structure of the nerve-cell and its processes. By 1891 the general anatomical relations of nerve-cells and nerve-fibres had been cleared up, largely through the brilliant work of Golgi and Cajal on the brain and spinal cord, and of von Lenhossék, Retzius, and others on the nervous system of annelids and other invertebrates. In these latter had been recognised the receptor cells, the motor or effector cells, and intermediary or internunciary cells interpolated between the receptors and effectors. In June 1891 Waldeyer put forward the neurone theory, the essence of which is that the nerve-cells are independent and that the processes of one cell, though coming into contiguous relation and interlacing with those of another cell, do not pass over into continuity. He founded his views partly upon evidence from embryological researches by His, but chiefly on results obtained from Golgi preparations and from anatomical investigations by Cajal.

The neurone theory aroused sharp controversy, and this stimulus turned many acute observers—zoologists and histologists—to the intimate study of the nerve-cell. First among the able opponents of the theory was Apáthy, whose well-known paper, published in 1897, on the conducting element of the nervous system and its topographical relations to the cells, first made known to us the presence of the neurofibrillar network in the body of the nerve-cell and the neurofibrils in the cell-processes. Apáthy held that the neurofibrillar system formed a continuous network in the central nervous system, and he propounded a new theory of the constitution of the latter, and was supported in his opposition to the neurone theory by Bethe, Nissl, and others. The controversy swung to and fro for some years, but the neurone theory—with certain modifications—seems now to have established itself as a working doctrine. The theory first enunciated as the result of morphological studies receives support from the experimental proof of a slight arrest of the nerve-impulse at the synapse between two neurones, which causes a measurable delay in the transmission.

The latest development in morphological work on nerve-elements is the investigation of the neuromotor system in the Protozoa. Sharp (1914), Yocom (1918), and Taylor (1920), working in Kofoid's laboratory, have examined this mechanism in the ciliates Diplodinium and Euplotes, and they describe and figure a mass—the neuromotorium—from which fibrils pass to the motor organs, to the sensory lip, and, in Diplodinium, to a ring round the œsophagus. The function of the apparatus is apparently not supporting or contractile, but conducting. By the application of the finest methods of microdissection, specimens of Euplotes have been operated upon while they were observed under an oil-immersion objective. Severance of the fibres destroyed co-ordination between the membranelles and the cirri, but other incisions of similar extent made without injuring the fibrillar apparatus did not impair co-ordination, and experiments on Paramæcium by Rees (1922) have yielded similar results. While the experimental evidence is as yet less conclusive than the morphological, it supports the latter in the view that the fibrils have a conducting, co-ordinating function. Progress in our knowledge of the nervous system is but one of many lines of advance in our understanding of the correlation and regulation of the component parts of the animal organism.

The ciliate Protozoa have been the subject during the last twenty years of a series of investigations of great interest, conducted with the purpose of ascertaining whether decline and death depend on inherent factors or on external conditions. While these researches have been in progress we have come to realise more fully that ciliates are by no means simple cells, and that some of them are organisms of highly complex structure. Twenty years ago Calkins succeeded in maintaining a strain of Paramæcium for twenty-three months, during which there were 742 successive divisions or generations, but the strain, which had exhibited signs of depression at intervals of about three months, finally died out, apparently from exhaustion. From this work, and the previous work of Maupas and Hertwig, the opinion became general that ciliates are able to pass through only a limited number of divisions, after which the animals weaken, become abnormal and die, and it was believed that the only way by which death could be averted was by a process of mating or conjugation involving an interchange of nuclear material between the two conjugants and resulting in a complete reorganisation of the nuclear apparatus. Jennings has shown that conjugation is not necessarily beneficial, that the ex-conjugants vary greatly in vitality and reproductive power, and that in most cases the division rate is less than before conjugation. Woodruff has since May 1, 1907, kept under constant conditions in culture a race of Paramæcium. During the sixteen years there have been some ten thousand generations, and there seems no likelihood of or reason for the death of the race so long as proper conditions are maintained. The possibility of conjugation has been precluded by isolation of the products of division in the main line of the culture, and the conclusion is justifiable that conjugation is not necessary for the continued life of the organism. The criticism that Woodruff's stock might be a non-conjugating race was met by placing the Paramæcia, left over from the direct line of culture, under other conditions, when conjugation was found to occur. Later observations by Erdmann and Woodruff show that a reorganisation of the nuclear apparatus of Paramæcium takes place about every twenty-five to thirty days (forty to fifty generations). This process, termed endomixis (in contrast to amphimixis), seems to be a normal event in the several races of Paramæcium which Erdmann and Woodruff have examined, and it is proved to coincide with the low points or depressions in the rhythm exhibited by Paramæcium.

Enriques (1916) maintained a ciliate—*Glaucoma pyriformis*—through 2701 generations without conjugation, and almost certainly without endomixis. From a single "wild" specimen he raised a large number and found that conjugating pairs were abundant, so that the objection could not be made that this was a non-conjugating race. Enriques then began his culture with one individual, and examined the descendants morning and evening, removing each time a specimen for the succeeding culture. The number of divisions per day varied from nine to thirteen, and as there was no break in the regularity and rapidity of division, and no sort of depression, Enriques concluded that neither endomixis nor conjugation could have occurred, for these processes take some time and would have reduced considerably the rate of division. These results, especially if they are confirmed by cytological study of preserved examples, show that for Glaucoma neither conjugation nor endomixis is necessary for continued healthy existence. Hartmann's observations (1917) on the flagellate *Eudorina elegans* extend the conclusion to another class of Protozoa. He followed this flagellate through 550 generations in two and a half years. The mode of reproduction was purely asexual, and there was no depression and no nuclear reorganisation other than that following fission. The evidence seems sufficient to confirm the view that certain Protozoa, if kept under favourable conditions, can maintain their vigour and divide indefinitely, without either amphimixis or endomixis.

Child (1915) states as the result of his experiments that the rate of metabolism is highest in Paramæcium and other ciliates immediately after fission—"in other words, after fission the animals are physiologically younger than before fission." This view, that rejuvenescence occurs with each fission, derives support from the observations of Enriques and Hartmann, for no other process was found to be taking place and yet the vigour of their organisms in culture was unimpaired. If, then, fission is sufficiently frequent—that is, if the conditions for growth remain favourable—the protoplasm maintains its vigour. If through changes in the external conditions the division rate falls, the rejuvenescence at each fission may not be sufficient to balance the deterioration taking place between the less frequent divisions. Under such conditions endomixis or conjugation may occur with beneficial results in some cases, but if these processes are precluded there is apparently nothing to arrest the progressive decline or "ageing" observed by Maupas and others.

The culture of tissues outside the body is throwing new light on the conditions requisite for the multiplication and differentiation of cells. R. G. Harrison (1907) was the first to devise a successful method by which the growth of somatic cells in culture could be followed under the microscope, and he was able to demonstrate the outgrowth of nerve-fibres from the central nervous tissue of the frog. Burrows (1911), after modifying the technique, cultivated nervous tissue, heart-cells, and mesenchymatous tissue of the chick in blood-plasma and embryonic extract, and this method has become a well-established means of investigation of cell-growth, tissues from the dog, cat, rat, guinea-pig, and man having been successfully grown. One strain of connective tissue-cells (fibroblasts) from the chick has been maintained in culture in vigorous condition for more than ten years—that is, for probably some years longer than would have been the normal length of life of the cells in the fowl. Heart-cells may be grown generation after generation—all traces of the original fragment of tissue having disappeared—the cells forming a thin, rapidly growing, pulsating sheet. Drew (1922) has recently used instead of coagulated plasma a fluid medium containing calcium salts in a colloidal condition, and has obtained successful growth of various tissues from the mouse. He finds that epithelial cells when growing alone remain undifferentiated, but on the addition of connective tissue differentiation soon sets in, squamous epithelium producing keratin, mammary epithelium giving rise to acinous branching structures, and when heart-cells grow in proximity to connective tissue they exhibit typical myofibrillæ, but if the heart-cells grow apart from the connective tissue they form spindle-shaped cells without myofibrillæ.

For many lines of work in modern zoology biochemical methods are obviously essential, and the applications of physics to biology are likewise highly important—*e.g.* in studies of the form and development of organisms and of skeletal structures. Without entering into the vexed question as to whether all responses to stimuli are capable of explanation in terms of chemistry and physics, it is very evident that modern developments have led to the increasing application of chemical and physical methods to biological investigation, and consequently to a closer union between biology, chemistry, and physics. It is clear also that the association of zoology with medicine is in more than one respect becoming progressively closer. Comparative anatomy and embryology, cytology, neurology, genetics, entomology, and parasitology, all have their bearing on human welfare.