BRITISH MEDICAL JOURNAL

LONDON SATURDAY JANUARY 14 1950

THE BEARING OF RECENT WORK ON THE VIRUS THEORY OF CANCER*

BY

C. H. ANDREWES, M.D., F.R.C.P., F.R.S.

National Institute for Medical Research, Mill Hill, London

Some neoplasms, for example a kidney tumour of leopard frogs and a series of sarcomata of fowls, own a virus as their causative agent. Filtrates of such tumours can be injected into other frogs or hens, as the case may be, and will reproduce histologically similar tumours in the inoculated species, and so on in indefinite series. With most animal tumours, however, filtrates are ineffective. It is supposed by what is called the virus theory of cancer that there is nevertheless only a quantitative difference between the fowl sarcomata and most new growths, that there are merely technical difficulties which prevent the filtration of, let us say, the propagable tumours of mice and rats, and that a virus is essentially concerned in the general run of cancers. The virus theory has had its ups and downs, mostly downs, for pathologists in general have not regarded it favourably. On the other hand, many bacteriologists, and particularly virus workers, are impressed by arguments in its favour.

A New Approach

The two Imperial Cancer Research Fund lectures preceding mine—that by Dr. Gye (1949) in March and that by Dr. Craigie (1949c)—have described a new approach to the problem in the Fund's laboratories. It seems possible that this work may open up a new chapter in cancer research. The College of Surgeons has asked me as a virologist to give this lecture, though I have not been personally concerned in cancer research for the last ten years. I have, however, previously worked upon and been deeply interested in the virus tumours for many years, and they possibly felt it would be useful for a virologist, standing on the side-lines, to say how the recent work struck him.

My own attitude to the virus theory is this: from the work of Rous, Gye, and others, and from my own, I have felt that there were very good reasons for exploring to the full the possibility that viruses caused all cancers. I have felt, for reasons I will explain, that the virus theory is intellectually satisfying. I do not consider it as proved, and am prepared to abandon it any day if shown good reason. I have just returned from the United States, where a reporter asked me my view on the virus theory. I replied that I thought it the best of the horses in the field, but that I would not, at this time, put my shirt on it.

The main reason for favouring the theory is that the virus is a good candidate for the post of a continuing cause

of cancer. Bashford, Rous, and lately Gye, in his lecture last March, have ably pointed out how cancer must have both remote and continuing causes. X rays, ultra-violet rays, chemical carcinogens, can all act to incite the beginning of a cancer, which may thereafter be carried on in indefinite series by means of cell grafts. But, having started the cancer off, they cease to operate, and some other cause must be found for the continuing reckless growth of the tumour. Viruses are known causes of cell proliferation, which may be evident over considerable periods of time, so the continuing cause might be a virus.

Another continuing cause could be a somatic cell mutation, and this, taken in a broad sense, is the main visible alternative to the virus theory. It can be urged that to evoke a somatic cell mutation is to tell what we already know—that a permanent change has taken place in a somatic cell when it becomes cancerous; we thus get no further. This is a little unfair, for we do come a little nearer to visualizing a possible mechanism of the change, which must involve a biochemical modification of a gene. It can be urged with greater force that of the hundreds of mutations known to be possible in the germ-cells of the fruit fly *Drosophila*—where their study has built up a science of drosophilology—none is known to lead to cancer. On the other hand, agents which induce mutations, such as x rays and radium, are known carcinogens. As a weapon of argument this is two-edged, for what can induce mutation in a mammalian cell might also do so in a virus within that cell. Mutation of somatic cells in a mammal apparently is a rarity. Yet from the nature of things it might frequently happen to a cell with restricted powers of multiplication and never be observed. There is, however, one kind of mutation which would, if it existed, necessarily force itself upon our notice, and that is a mutation of somatic cells which led to unrestricted cell proliferation. The main objection to the somatic cell mutation theory of cancer is that it rests on pure speculation; there is no concrete evidence, as there is for the virus theory, that it has ever actually occurred and given rise to a cancer. Nevertheless some pathologists will continue to favour it, just as others will incline to reject it, until decisive evidence one way or another is forthcoming. The virtue of the recent Cancer Research Fund work is that it seems to offer a hope of deciding between the two alternatives of the continuing cause of cancer—an agent, most probably a virus, separable from the cell, or a change, a mutation perhaps, involving one or more genes, necessarily happening within a cell and not separable from the whole cell.

*An Imperial Cancer Research Fund Lecture delivered at the Royal College of Surgeons on November 29, 1949.

Before turning to this recent work I must explain in some detail why I describe the virus theory as intellectually satisfying. (I hasten, however, to say that what is intellectually satisfying to one pathologist is not necessarily true.) I have worked with viruses for more than 25 years, and the more I learn of them the more am I impressed with the variety of the virus-host relations which can be found. On the one hand are the viruses which attack, invade, and overwhelm their hosts in a matter of days or even hours: yellow fever and influenza and many other viruses can do this. At the other extreme is the bacteriophage in a lysogenic bacillus, causing no visible harm to its host and to be detected only by its power to multiply in and lyse some other species of bacterium—a so-called indicator organism—if this is presented to it. In the first case the virus multiplication goes on unchecked until so many essential host cells are killed that the host cannot survive. In the latter case the bacterial virus probably multiplies *pari passu* with the bacterial host, no faster and no slower, being carried along to each daughter cell as the host practises binary fission after the manner of its kind.

The causation of cancer could be but one of the numerous manifestations of host-virus relations. What properties would a virus have to possess to enable it to fulfil this role? It would have to be capable of inducing cell proliferation; it must, as we shall see, be very plastic and variable; it must be transmissible very early in life or even in the germ-plasm; it must normally exist as an inapparent or latent infection, being tuned to the rate of multiplication of its host cell so that it neither overgrows and kills it nor itself becomes overgrown and lost; it must be almost ubiquitous; it must be capable of being activated by an upset to the host-parasite equilibrium; it must be non-contagious in the ordinary sense. Let us consider these properties in turn and see whether they are known to occur among viruses.

Cell Proliferation

Apart from the viruses causing malignant tumours, such as that of Rous fowl sarcoma, many cause the cells they infect to proliferate for a time. Vaccinia does so, and so to an even greater degree does fowl-pox; but with them, as with most viruses, cell multiplication is inevitably followed by cell necrosis. Shope's rabbit fibroma produces massive swellings due to multiplication of fibroblasts, and might well pass for a sarcoma but for the fact that the lesions ultimately regress. While I would not urge that the Shope fibroma be considered a true neoplasm, it is worth mentioning that growths produced in ducks by fowl tumour viruses are also likely to disappear completely. The infectious warts of man, rabbits, and other species are innocent growths in which cell proliferation may continue for many months. Domestic rabbits can be infected with Shope's papilloma virus, though this is native to wild cottontail rabbits. In the tame rabbit, less often in the cottontail, the warts may fail to regress but continue to a malignancy which finally kills the animal with metastasizing growths.

The relation between the papilloma virus and the resulting epitheliomata is obscure: the virus infection certainly acts as a remote cause of the cancers, but the virus cannot be directly recovered from them—indeed, it may be impossible to obtain it even from the papillomata of which it is unquestionably the continuing cause. When the cancers are grafted into other rabbits, these develop neutralizing antibodies against the papilloma virus, thus showing that the virus is still present in the growths, perhaps in masked

form. If, however, the grafting is continued long enough even this indirect method of detecting the virus's presence may fail. One can argue, quite reasonably, that the virus has disappeared and is therefore not the continuing cause of the cancers. It is, however, also possible that the timing between division of cell and division of virus has become perfectly synchronized, so that excess virus is no longer available to stimulate antibody production.

Variation

Viruses are so notoriously able to vary or mutate that this property needs little emphasis. One instance of this is the adaptation of a parasite of one species to growth in another—a plasticity of which virus workers take advantage every day; or the change in a virus to an affinity for one system, such as the central nervous system, instead of another—yellow fever and dengue viruses can be thus modified. Influenza virus is certainly capable of variation in its antigenic make-up. Of special interest is a change in the virulence of a virus for one and the same tissue. Thus the normal form of Shope's rabbit fibroma causes cell proliferation, but there is a variant which causes almost wholly inflammatory lesions (Andrewes and Shope, 1936). Probably the variant virus multiplies more rapidly and kills the cells instead of going along with and gently stimulating them, and the inflammation is the reaction of the tissues to the destroyed cells.

When we consider presently how various stimuli may upset a cell-virus equilibrium it will be obvious that a permanent change has often been induced by the upsetting stimulus. If the result should be a cancer, it would seem likely that the stimulus had acted by causing a mutation or variation of the hitherto latent virus or by so setting the stage that a particular mutant must emerge and dominate the scene.

One of the difficulties in the way of accepting the virus theory is a natural reluctance to believe that different histological types of tumour in different species can be due to different viruses, each causing a particular type of histological change in each kind of tissue of each species of animal. The number of cancer viruses necessary would be almost infinite. Fortunately such an imaginative feat is not required of us. One of the established facts of cancer research is that mammary cancers on continued transplantation may induce malignant transformation of their supporting stroma. It thus seems possible that there may appear a variant of an epitheliotropic virus having the power to infect connective tissue. There is also on record important work by Duran-Reynals (1942). He worked with the fowl-sarcoma virus of Rous, and this, in the fowl, is a stable virus, always producing tumours of the same general histological type with variations due merely to differing rates of growth. From chicks of a particular age group he was able to obtain Rous virus which could be adapted to grow in ducklings. The tumours he obtained were—and this is the important point—not all like the Rous fowl sarcoma, but showed considerable variation in histological pattern though all were connective-tissue tumours. Spontaneous duck tumours are almost unknown, so that the causation of Duran-Reynals's duck tumours can safely be attributed to the Rous virus. After adaptation to ducks it needed again a good deal of persuasion to induce the virus once more to infect chickens, and again some histological diversity was encountered. It seems as though when an established equilibrium is upset the virus may enter a plastic state. If such were the case one of the great objections to the virus theory would be overcome, for the

existence of infinitely numerous tumour viruses would not have to be envisaged.

Early Transmission

The virus of herpes simplex has been shown to be transmitted most usually to and from young children, giving rise to herpetic stomatitis. Those thus infected are commonly subject to herpes simplex or fever blisters in after-life, but those who escape infection in childhood apparently acquire a measure of non-specific resistance and may never be infected at all. So, too, psittacosis infection is commonly acquired by baby budgerigars as a nest infection, persisting in a latent form and perhaps never causing any trouble (Meyer, 1942). The Bittner virus of breast carcinoma in mice is, as is well known, commonly transmitted to sucklings in their mother's and foster-mother's milk.

Such instances could be multiplied many times, but what is more important is to consider the possibility of infection of cells with a virus before birth. Such must surely happen with some tumour viruses if viruses are to be postulated as concerned in all tumours; for Rous was able to induce cancers of various sorts in *embryonic* tissues of mice by treating them with methylcholanthrene (Rous and Smith, 1945).

The virus of lymphocytic choriomeningitis is one known to be capable of infecting baby mice *in utero*; indeed, with one strain of mice Traub (1939) found that such infection was the rule, all mice born of the infected mothers having a latent infection. One may ask also whether the congenital abnormalities which occur in children whose mothers have rubella early in pregnancy may not be due to *in utero* infection with that virus.

If we turn to arthropods we find that the virus of St. Louis encephalitis is carried in or on the ovum in the chicken mite *Dermanyssus gallinae*—a fact which explains the mechanism, hitherto a mystery, by which it was carried along through the winter months. Viruses affecting caterpillars of certain moths are also thought to be carried in or on the ovum or sperm.

An interesting example is afforded by scrapie, a virus disease affecting the nervous system of sheep and causing them to itch violently, lose their wool by scraping against posts, and often ending in incoordination, convulsions, and coma. The incubation period of the disease after inoculation is two years. Apparently a so-called scrapie ram can transmit infection to his offspring, though these remain unaffected till 2 years old. The ewe, their mother, may remain unaffected and bear healthy lambs by another ram. Transmission by the sperm is indicated in this disease also. Congenital infection with a virus seems thus to be a known phenomenon.

Inapparent Infections

Latency of virus infection is also so frequent as to be a commonplace. When we know more we may discover that virus infections are latent more often than they are overt, just as saprophytic bacteria are far more numerous than pathogenic ones. We have already had occasion to mention several examples. Herpes simplex lies latent in a majority of human beings, lymphocytic choriomeningitis in all mice of certain stocks, psittacosis and related viruses in many colonies of budgerigars and pigeons; scrapie is latent in the scrapie rams. So, too, a plant virus is latent in every King Edward potato, and phages lie hidden as unsuspected inhabitants of many apparently normal bacteria—so-called lysogenic bacteria. The Bittner virus may be for ever concealed and harmless in the mice carrying it.

Ubiquity of Viruses

In many of the above instances—herpes, psittacosis, choriomeningitis, and the lysogenic bacteria and King Edward potato—the virus may be present in almost every individual of certain strains or stocks.

Activation of Latent Viruses

The equilibrium between latent viruses and their hosts may be upset in many ways, the result being manifest disease. An intracerebral injection of broth may produce lymphocytic choriomeningitis in a carrier mouse. Overcrowding and faulty management may produce a crop of psittacosis cases in the budgerigar colony. The stimuli which may bring out a crop of fever-blisters in a so-called "habitual herpetic" are many and various: ultra-violet light, artificial heat, fever, and colds are among them. Chronic latent encephalitis may be set up in rabbits with herpes virus, and sublethal anaphylactic shock is particularly effective in activating this—a possible clue to the mechanism of the action of some of the agents effective in starting off herpes blisters in man. It is of some interest that Shope's rabbit fibroma—which is a benign and local though not exactly latent infection—is rendered much more active and becomes generalized when the infected rabbits are injected with tar or carcinogenic hydrocarbons (Ahlström and Andrewes, 1938). Time and experience will show whether this finding has any bearing on the power of carcinogenic chemicals to evoke cancers, whether in fact they can perchance be acting by upsetting an equilibrium between a tumour virus and its host cell.

Transmissibility

Whether or not cancer has a virus as a factor in its causation, it is certainly not an infectious or contagious disease. Neither are the acknowledged virus-tumours, the fowl sarcomata. Nor is herpes simplex in adults, or only very feebly so. Nor is St. Louis or equine encephalitis as it occurs in man or the horse. Nor is pseudo-rabies in cattle. Nor are any of the many virus diseases which are rare highly conditioned manifestations of a virus infection which is very widespread. Ready transmission of a virus disease happens very often, but often it does not.

It will now, I hope, be clear why I find the virus theory of cancer satisfying, for a cancer virus fits quite comfortably into the body of facts we already know concerning viruses and their varied habits. There is, however, the undeniable fact that the virus theory is only a theory and that it cannot be regarded as proved in face of an inability to find, in cancers in general, positive evidence for the existence of a continuing cause apart from the cell itself. Most transmissible tumours of mammals are *not* filterable like many fowl sarcomata, and have not hitherto been transmitted otherwise than by cell grafts.

Two Recent Lectures

In this context must be read, or have been heard, the two recent Cancer Research Fund lectures by Drs. Gye (1949) and Craigie (1949c). Here are definite suggestions that the widely studied cancers of mice may not, after all, be essentially different from the virus tumours but like them transmissible by means of something other than the living cell itself. Dr. Gye and Dr. Craigie will, I trust, forgive me if I act as devil's advocate and examine their evidence critically in order to determine whether or not we are in a position to regard the virus theory as being proved or

even substantially buttressed by the recent work. Their approaches are very different. Dr. Gye has long been an advocate of the virus theory and feels that recent findings finally establish its truth. The point at issue is, however, so fundamental in cancer research that the many who dislike the virus theory—and they are not necessarily diehards or fools—will require proof of unusual rigidity before they will accept his conclusions. Dr. Craigie's approach is more cautious: he is concerned to establish new and better quantitative methods for attacking the problem and eventually to see where this line of attack has brought him as regards the truth or falsehood of the virus theory.

The pith of the matter is whether mouse tumours can be transmitted by agents other than intact cells. Filtration would be the most certain method of proving this. But with viruses of low titre or great lability filtration may fail and recourse must then be had to other techniques. Dr. Gye raises the question of whether living cells can survive fine mincing and suspension in media containing glucose, whether they can withstand freezing and above all desiccation. As regards the freezing of suspensions of minced tumours, he writes: "The results cannot be interpreted simply as being the consequence of survival of cells on freezing"; and, again, "It is in the highest degree improbable that the tumours are a mere grafting of living cells." He admits that there is some doubt in the matter. Miss Mann (1949a), however, writes: "By freezing mammary carcinoma tissue at -79°C . for long periods the tumour cells are killed." Much evidence suggests that this is so, but many will feel that rigid proof is lacking. Miss Mann (1949b) finds that minced mouse embryo will form "large embryomas which persist for months or indefinitely" when grafted into homozygous mice. Such embryo-mince, unlike tumour tissue, is rendered ineffective by freezing. There are, however, no data on the relative dilutions to which embryo-mince and tumour-mince respectively can be subjected without loss of potency. There is not excluded the possibility that freezing reduces both alike by 99%. It is known that skin grafts can be kept frozen and used successfully. Craigie quotes Briggs and Jund (1944) in this connexion. Klinker (1937) claims to have obtained growth of tissue cultures from frozen tissue, though others have failed. To say the least, there is this conflict of opinion on how far freezing kills mammalian cells.

The dextrose which Craigie (1949b) uses to preserve his cell suspensions causes profound alterations in nuclear structure as seen with the phase-contrast microscope. One could be pardoned for presuming cell death when seeing such changes; these are, however, reversible when sodium chloride is added, so one is in doubt what to think.

All are agreed that a mammalian cell completely dried is much less likely to be alive than one frozen or treated with dextrose. Gye naturally regards experiments with dried tumour tissue as more conclusive than those where other methods of treatment are used. Craigie (1949a) has described the drying-plant which made possible the propagation of tumours with the aid of dried tissue, and his first successful results with a mouse sarcoma. He obtained, but has not yet published, quantitative information indicating the relative activity of the fresh and dried tissue.

Points on Drying

Accounts of successful drying of two other mouse sarcomata and three mouse carcinomata are less detailed as yet in that they lack such full quantitative data. The available evidence indicates that drying has been very complete, perhaps more complete than the standard lyophilization used

by workers with bacteria and viruses. Three points, however, need comment. The drying has been done in most experiments in the presence of dextrose, the water of crystallization of which has not been removed, or of glycerin. Ignorance of what could happen to a cell dried in such circumstances introduces an unknown element into our appraisal of the results. Secondly, the dried tumours have as yet been tested immediately, without any storage in the dried state. Thirdly, in Dr. Gye's lecture and Miss Mann's paper (Mann and Dunn, 1949) much emphasis is placed on the use of a novel principle—the holding of tumour for some time in the frozen state before drying. The results certainly show better survival in the experiments where this was done—an unexpected and unexplained finding. The point may be as important as the authors believe, but the published data lack adequate evidence that the same tumour specimen dried after storage at -79°C . gave better results than when dried from the fresh state. More *ad hoc* experiments are necessary before the importance of the steps can be rightly evaluated.

The Histological Approach

Histological evidence indicates that the dried yet still potent tumour tissue contains no living cells. This, when cleared, embedded, and cut without rehydration, has an appearance very unlike that of living cells. True, the grosser cell-structure is largely restored on rehydration, but finer structure remains much impaired. The histological approach, however, has the grave disadvantage that an odd living cell here and there might escape the most careful observation. Most pathologists, asked whether mammalian cells could survive desiccation, would say "No," but others would reply "Why not?" particularly if the alternative was an admission that cell-free transfer of malignancy was established. Survival of dried bacterial cells is a commonplace, and, lately, spermatozoa have been found able to wiggle their tails after not quite complete drying in the presence of glycerin. Histology, argument, or preconceived ideas cannot settle the matter. The important question has to be decided by other means.

One obvious approach is to see whether a dried carcinoma will "take" when introduced somewhere where it has no access to the appropriate epithelial cells. An epitheliotropic carcinoma-causing virus would not be expected to produce tumours if it had access only to connective tissue: carcinoma grafts could of course, and commonly do, grow in such situations. If a dried carcinoma were able to grow in these circumstances, that fact would suggest that intact cells were still present. Unfortunately the most readily available mouse carcinomas are mammary growths, and mammary tissue is present almost everywhere in a mouse's subcutis. Rudiments are present even in males. Conclusive evidence would be likely to come from study of carcinomas of epithelium other than that of the mamma. Miss Mann (1949a) sought to settle this matter by injecting frozen mammary cancer tissue intraperitoneally, and finds that, while it takes well subcutaneously, it fails to take in the peritoneum except in odd instances. An occasional carrying-in of a little breast tissue on the needle is invoked to explain this—and not unreasonably. The unfrozen grafted tumour is reported to take both intraperitoneally and subcutaneously. Unfortunately, the experiment is inconclusive, for we are again not told at what dilutions the fresh and frozen tissues respectively would give takes by the two routes of injection. Without this knowledge we cannot interpret the result.

Grafts of the tumours so far studied by Gye and his colleagues grow best, or exclusively, in homozygous mice.

No details are yet available about whether the frozen or dried tissue is equally dependent for successful growth on being injected into a mouse of the same genetic constitution. If it were not, there would be good reason for thinking that cell-grafting was no longer wholly the mode of transmission. If it were still necessary, we should have to believe that we were dealing still with grafted cells or else with a virus having a novel type of specificity—not merely for a particular species, but for individuals within that species of a particular constitution; this would indeed be novel but by no means impossible.

I must mention in passing that some would like to reconcile the virus theory with other theories of cancer by supposing that an inheritable cellular change is always involved, but that in some exceptional instances, as in fowl sarcomata, a self-replicating piece of the altered cell, a plasmagene, or what-you-will, becomes free and able to affect other cells; that, in fact, a tumour virus when demonstrable is a product of the attacked cell and not an autonomous parasite. This to me is a hypothesis with almost nothing in its favour; it is gratuitously unnecessary. If there is a free active agent concerned, I prefer to think of it as an honest-to-goodness virus, an independent parasite like other viruses, and I have already tried to show that its properties would not greatly differ from those of other viruses. Heterogenesis, the doctrine that living things may arise from something utterly unlike themselves, as flies from putrid meat, has been propounded again and again; it has always proved wrong, and will again.

Conclusion

My lecture may have seemed to be arguing, as to its first half, for accepting as feasible the virus theory of cancer and, as to its second half, for doubting a lot of the evidence put forward in favour of that theory. That is not an illogical position. I confess that I should feel, if the virus theory were proved true, that the world was a more orderly place and that all was ready for important advances in the cancer field, going on from the newly consolidated ground. That is the very reason why I feel such an impelling need for confidence that the ground is truly solid and that this can never shift from under our feet. It would be of first-class importance to establish certainly that mouse cancers in general—and not one or two abnormal growths—could be transmitted otherwise than with intact cells. The new techniques Dr. Craigie has described open the way to settling this matter. There are so many things crying out to be done that I am sure we shall learn much more very soon. But—*festina lente*! If I cannot be very positive and dogmatic to-day, let it be remembered that I have been trying to review the present position of the virus theory at a very difficult time, when a new attack on the problem is just being launched.

REFERENCES

- Ahlström, C. G., and Andrewes, C. H. (1938). *J. Path. Bact.*, **47**, 65.
 Andrewes, C. H., and Shope, R. E. (1936). *J. exp. Med.*, **63**, 179.
 Briggs, R., and Jund, L. (1944). *Anat. Rec.*, **89**, 75.
 Craigie, J. (1949a). *Brit. J. Cancer*, **3**, 250.
 — (1949b). *Ibid.*, **3**, 268.
 — (1949c). *British Medical Journal*, **2**, 1485.
 Duran-Reynals, F. (1942). *Cancer Res.*, **2**, 343.
 Gye, W. E. (1949). *British Medical Journal*, **1**, 511.
 Klinker, J. (1937). *Z. Krebsforsch.*, **46**, 436.
 Mann, I. (1949a). *British Medical Journal*, **2**, 251.
 — (1949b). *Brit. J. Cancer*, **3**, 255.
 — and Dunn, W. J. (1949). *British Medical Journal*, **2**, 255.
 Meyer, K. (1942). *Medicine*, **21**, 175.
 Rous, P., and Smith, W. E. (1945). *J. exp. Med.*, **81**, 597.
 Traub, E. (1939). *Ibid.*, **69**, 801.

AGE OF PUBERTY IN THE TROPICS

BY

RICHARD W. B. ELLIS, M.D., F.R.C.P.

Professor of Child Life and Health, University of Edinburgh

The belief is widely held that children mature earlier in tropical than in temperate climates. This view has not only been repeated by medical authorities but in some instances is reflected in local legislation relating to age of consent and marriage and to the protection of juvenile labour. In actual fact, however, such evidence as exists points to a later rather than an earlier age of menarche (first menstruation) in girls living in tropical areas. No statistical studies of boys appear to have been made. Mills (1937), from a study of the mean age of menarche and the presumptive period of adolescent sterility following the menarche, states: "Sexual maturity in tropical countries comes fully two years later than in the most stimulating temperate regions."

While most recent studies have lent general support to Mills's view that in a tropical climate the menarche is retarded rather than accelerated (Howe, 1939; Ito, 1942; Kark, 1943), and Ogle (1934) has shown that a hot moist atmosphere may retard sexual maturity in experimental animals when the diet is controlled, it is clear that the available evidence is still rather meagre and much of it open to criticism. This is understandable when it is remembered that in many communities living in tropical areas knowledge of exact age is exceptional and memory of age of menarche correspondingly unreliable. It is also impossible in most instances to assess the relative importance of socio-economic conditions, including diet, and of climate when comparing subjects living in tropical or temperate regions. Heredity, on the other hand, whilst it may affect the age of puberty of the individual, is probably not of major importance when communities of different ethnic stock living under similar social and environmental conditions are compared (Hogben *et al.*, 1948; Michelson, 1944).

The following investigation was carried out in Nigeria during the rainy season, July to September, 1949, and comparisons made with figures previously obtained in Great Britain for both girls and boys (Ellis, 1947). The great majority of the subjects included in the study were children born and educated in Lagos, where births have been registrable long enough to allow verification of age; a smaller proportion were children from mission schools elsewhere in Southern Nigeria whose age was accurately known. None of the children had been out of Nigeria, and no European children were included. Although no attempt has been made to classify the children by tribes, the great majority were Yoruba or Ibo. Since school-children in Nigeria represent a privileged section of the community, it may be assumed that the nutrition of those included in the survey would be above the average of children in the southern provinces generally, where probably less than 20% of girls of school age are in fact attending school.

The Southern Nigerian diet is largely vegetable (yams, cassava, cornflour, plantains, etc.) and includes little meat and practically no milk. Fish, though expensive, is more readily obtainable in Lagos than in most inland areas. Red palm oil, which is widely used, provides a rich source of vitamin A and palm wine of vitamin B complex. A considerable number of the Lagos children were receiving a mid-day meal at school, which usually meant that groundnuts