Papers and Originals

New Growth and Viruses*

MICHAEL STOKER,† M.D., F.R.S., F.R.S.ED.

British Medical Journal, 1970, 3, 541-545

To members of the medical profession new growth is one of the synonyms for cancer, and in this lecture I shall be dealing mainly with cancer and the viruses which induce it. The title is chosen to emphasize the word "growth," however, because I want to discuss some aspects of growth regulation in individual normal cells and the perturbations which occur in cancer cells.

Long-range and Short-range Regulation of Growth

The growth of a living cell is regulated by the external environment, and for multicellular organisms such as man this means not only the extracellular fluid surrounding the cells but the surface of other cells in the immediate proximity. Many types of cells cease to grow at a stage immediately after mitosis but before onset of D.N.A. synthesis (the G1 phase), but they retain the potential to grow again, if required, during the life of the individual. Whether growth starts or not at a particular time and place must depend on a variety of brakes and accelerators included in feedback circuits, of which we are almost entirely ignorant. We may suppose, however, that they will fall into two main classes. First there will be long-range effects, presumably caused by fairly stable molecules produced by cells far removed from the target cell and circulating in blood and tissue fluid. Some familiar hormones are included in this class. Second, there will be short-range effects, whereby cells influence one another over short distances, perhaps a few cell diameters. These might operate by direct contact between cell membranes or by diffusion gradients over short distances. It is probably short-range information, available to a cell from its immediate surroundings, which allows it to know not only its own address but whether or not it should multiply, move, or differentiate to maintain a characteristic histology.

Cell Specificity of Control Systems

The short-range and long-range regulating mechanisms may be of quite different types. Cell specificity should be more important for some of the long-range growth factors, because the molecules concerned, whether growth promoters or inhibitors, have to find the right target cells. For example, erythropoietin causes erythroid but not granulocyte precursors to divide. Oestrogen (without progesterone) stimulates the uterine epithelium but not stroma (Martin and Finn, 1968).

In recent years there has been growing interest in the idea of long-range tissue-specific inhibitors, or "chalone," released by cells in particular tissues and active only on cells in the same type of tissue. This was suggested originally by the ingenious experiment reported by Bullough and Laurence

(1960), where removal of epidermis on one side of a rabbit's ear led to increased mitosis at the edge of the wound and also in the epidermis on the opposite side of the ear, but not in the intervening dermis. They suggested that this was the result of the loss of specific inhibitory molecules (epidermal chalones) from the area. Subsequently, extracts which inhibit mitosis in the tissue of origin have been obtained not only from skin but from other tissues such as liver, lung, kidney, and also granulocytes (see review by Bullough, Laurence, Iversen, and Elgjo, 1967). These extracts are not species-specific, and their activities are enhanced by stress hormones such as adrenaline. Conclusive evidence that chalones are tissue-specific, however, still depends on more convincing experiments to show lack of reciprocity of action of two types of chalone in the same experimental system. This type of experiment and the purification and characterization of chalones is eagerly awaited.

By contrast, control at short range does not require high specificity. For example, one small group of cells may be induced to divide by interaction with a common neighbour involving quite non-specific factors.

Growth Regulation in Cell Cultures

Because of the forbidding complexities which arise from the integration of many regulation systems, within and between a great variety of cell types in whole animals, many workers have turned to the study of growth control in cultured cells; investigations are generally made with fibroblasts because they are easy to manipulate. Fibroblastic cells from normal embryonic or adult tissues may be used, but they are a heterogeneous lot, and since the emphasis is on simplification and reproducibility it is an advantage to use homogenous populations cloned from single cells. This generally means using stable cell lines. Many stable lines resemble tumour cells, but there are several, such as the 3T3 mouse cell line (Todaro and Green, 1963) and the BHK 21 hamster cell line (Macpherson and Stoker, 1962), which retain certain characteristics of normal cells, and, most importantly, they can be transformed into tumour cells by viruses.

Over the last two decades excellent methods have been developed for ensuring the rapid proliferation of cells in culture, so the first problem is to find ways of inhibiting cell growth without causing damage and thus to mimic the quiescent state of most cells in the adult organism. It calls for a reversible and a reasonably physiological inhibition in the G1 phase of the cycle preferably subject to disturbance by carcinogens.

Density and Wounding

One of the most interesting forms of growth inhibition is that which occurs at high cell density, sometimes called contact inhibition of growth, and not to be confused with contact inhibition of movement (Abercrombie and Heaysman, 1954). In a given medium cells grow on a glass or plastic surface to

* Annual Scientific Address given at the Royal Postgraduate Medical School of London on 15 May 1970.

† Director of Research, Imperial Cancer Research Fund Laboratories, London W.C.2.

form a confluent sheet, and then they stop growing in the G1 phase unless the density is reduced. Some cells, such as the 3T3 cell line, are inhibited at fairly low density when they first make general contact with one another; others such as the BHK 21 line of cells, grow to form several layers and then stop. Though exhaustion of growth factors may contribute, it cannot completely explain this inhibition, because the medium from dense non-growing cultures is usually adequate for growth of cells at lower density. This can be dramatically shown by "wounding" a sheet of dense cells without changing the medium, by wiping part of the cell sheet away and leaving a bare area and a sharp edge to the remaining cell sheet (Todaro, Lazar, and Green, 1965). Cells migrate from the dense area to the bare area where onset of growth may be observed by uptake of tritiated thymidine and by mitosis.

An analysis of the history of individual 3T3 cells by time-lapse cinematography showed that D.N.A. synthesis followed about 20 hours after the cells began to move from the edge of the dense inhibited sheet, but the critical event was a reduction in the degree of contact with other cells (Dulbecco and Stoker, 1970). If this occurred, whatever the subsequent history, D.N.A. synthesis followed in about nine hours. These experiments did not show if loss of contact had an initiating effect on its own or whether it operated indirectly—for example, by affecting uptake of an essential medium factor. But, whatever the mechanism, there is no evidence of cell specificity, at least between normal cells, because the inhibition occurs as well among mixed cultures of different cell types as in pure cultures (Levine, Becker, Boone, and Eagle, 1965). It is an interesting example of short-range regulation and may obviously be relevant to the problem of wound regeneration *in vivo*.

Anchorage

Another method of inhibiting growth of fibroblasts also in the G1 phase is by forbidding anchorage to a rigid surface (Stoker, O'Neill, Berryman, and Waxman, 1968). Cells suspended in viscous growth medium containing agar or methyl cellulose remain healthy but do not grow. If, however, they are allowed to touch a rigid surface of sufficient size (about the area of one spread cell) by falling on to glass or plastic or by introduction of foreign bodies into the medium, they attach and spread, and the growth cycle begins first with D.N.A. synthesis and then division. Again this could be the direct effect of a cell membrane change on an initiation step, or it could be caused by altered uptake or excretion affected by cell shape or interaction with the surface. Whether this phenomenon is relevant to the intact organism is unknown; most fibroblastic cells are probably anchored, but to rather elastic structures such as collagen.

Serum Factor

We must now consider the role of growth factors in cell cultures. Fibroblasts in culture will not multiply in defined synthetic medium containing essential amino-acids, vitamins, salts, etc. For growth they require naturally occurring substances which are most conveniently provided experimentally by adding serum (Healey and Parker, 1966; Bürk, 1967; Todaro, Matsuya, Bloom, Robbins, and Green, 1967; Holley and Kiernan, 1968; Temin, 1968). Though serum undoubtedly contains many substances which affect cells, there is one particular factor (or group of factors) which is required for initiation of D.N.A. synthesis, the first step in the cell growth. It is protein, associated with the gammaglobulin fraction, and is at present referred to simply as serum factor, even though it may be due to more than one type of molecule. There is so far little evidence suggesting that serum factor is species or cell type specific. Sera of calves, fetal calves, humans, other mammals, and birds, for example, all induce the growth of hamster

and mouse fibroblastic cells. Serum factors have not been completely replaced by any of the human hormones, though preparations of insulin thyrotrophic and gonadotrophic hormone can stimulate growth under certain conditions (Temin, 1967; Holley and Kiernan, 1968).

In the absence of serum factor cells remain stationary in the G1 phase of the cycle. Exposure to serum leads to D.N.A. synthesis after about 10 hours and subsequently to mitosis. The induction of D.N.A. synthesis occurs on an all-or-none basis in any individual cell, and, therefore, a count of the number of cells incorporating thymidine provides a convenient assay of the activity of the serum factor.

Changes in Sensitivity to Serum Factor

Using this assay, we have studied the serum factor requirements of BHK 21 cells in different conditions of density and anchorage, to search for common features of these various control systems (Clarke, Stoker, Ludlow, and Thornton, 1970). It was already known that resting confluent cultures of 3T3 cells could be made to grow further by adding more fresh serum (Todaro, *et al.*, 1965). We found that resting BHK 21 cells also responded to serum whatever their density, state of suspension, or anchorage, but the *probability* that a particular cell would respond to a given dose of serum by commencing D.N.A. synthesis was markedly affected by the position of the cell in relation to other cells. This is shown in Fig. 1 by comparing the response to varying amounts of serum of the undisturbed cell sheet with the cells emigrating into wounds in the same sheet. In both situations cells require serum for initiation of D.N.A. synthesis, but a free cell in the wound is some eight times more sensitive than an undisturbed cell surrounded by other cells in the dense part of the culture. Similar results with BHK 21 cells have been reported by Dulbecco (1970) in a comparative study of different cells responding to wounding.

We have also found that the growth cycle can be induced even in suspended cells if they were given high enough doses of serum, but a comparison of the response curves for suspended and anchored cells shows that anchorage greatly increases the probability of response. These changes in response to serum are shown diagrammatically in Fig. 2.

To sum up, therefore, we can now envisage a control system for fibroblasts based on response to one or more common initiation factors, normally available in the extracellular environment but with a specificity of action determined by the state of the cell. Whether a particular cell divides will depend not only on the concentration of the factor in the environment but on topographical considerations—that is, the

FIG. 1.—Initiation of D.N.A. synthesis in resting BHK cells after wounding. In replicate confluent cultures part of the cell layer was wiped off to give a free edge. Cultures were then exposed for 20 hours to the serum concentration shown and to ^3H thymidine at 2 μC . per ml. (23.5 c. per mMole) before autoradiography. The figure shows the percentage of cells incorporating thymidine in the undisturbed confluent layer and in the zone of cell emigration at the edge of the wound.

FIG. 2.—Schematic representation showing the influence of cell morphology and contact state on the initiation of D.N.A. synthesis in normal BHK cells by serum. From left to right: suspended cells, anchored cells in contact, anchored cells not in contact.

position of the cell in relation to other cells and surfaces—and perhaps on the shape of the cell. Whether these changes operate by altering the cell surface is at present unknown. It is also impossible to say whether this system observed in cell culture has any relevance to the control of cell growth in the intact organism, but it shows how local regulation can be superimposed on a general system involving widely distributed growth factors.

Viruses and Cell Growth: Non-cytocidal Infection

I wish now to turn to the effect of viruses, particularly tumour viruses, on the growth regulation systems which have been discussed. Most of the more familiar viruses, such as poliomyelitis, kill the cells in which they multiply. Such infections are called “lytic” or “cytotoxic,” and are of little interest to us here because a dead cell cannot give rise to a tumour. Tumour viruses, however, have the property of infecting cells without killing them, and changes in function of the surviving cells may then follow.

Non-cytocidal infections by tumour viruses occur in two ways. Some viruses grow and leave the cells by budding from the surface without disrupting them. This is true of all the R.N.A.-containing tumour viruses such as those causing leukaemia and some types of sarcoma in animals. Multiplication of the D.N.A.-containing tumour viruses, such as polyoma, SV40, and adenoviruses, is inevitably cytotoxic, however, perhaps because virus assembly takes place in the nucleus. Some of the early changes which we believe to be characteristic of tumour cells—stimulation of cell D.N.A. synthesis, for example—are detectable before the cell dies (Dulbecco, Hartwell, and Vogt, 1965; Weil, Michel, and Ruschmann, 1965; Winocour, Kaye and Stoller, 1965). The appearance of living tumour cells, however, can only follow incomplete virus infections in which some of the virus genes do not function, in particular those responsible for the later stages of virus growth leading to cell death.

Such incomplete infection may occur if there is a mutation affecting these “late” virus genes, or by infection of certain types of host cell, which permit the early virus genes but not

the late genes to function. Such cells are called non-permissive, in contrast to the permissive cells in which all virus genes are expressed, the virus replicates, and the cells are killed.

Viral Transformation

Any non-cytocidal infections by tumour viruses may lead to the appearance, among the survivors, of cells with permanently altered behaviour, in particular altered growth potential. These are called transformed cells, and they develop into clones which can easily be recognized in the cultures. Transformed cells have many but not necessarily all the characteristics of cells obtained from tumours induced in vivo. We believe viral transformants represent the initial change to malignancy that occurs before progression takes place by successive emergence of cell variants during the course of tumour growth. Though tumour viruses can only transform cells as a result of non-cytocidal virus infection, it should be remembered that viruses capable of non-cytocidal infection are not necessarily tumour viruses. Rabies virus, for example, grows without killing cells and leads to change in cell function, with quite different manifestations. Other viruses may grow as silent commensals without otherwise detectable change in the cell. Transformed cells continue to carry the viruses which initiated the transformation; cells transformed by R.N.A. viruses, for example, continue to release new virus particles. In cells transformed by D.N.A. viruses complete virus synthesis cannot normally be detected, and would anyway be cytotoxic. The viral genome—that is, the viral D.N.A.—can, however, be detected by chemical or genetic procedures, and the evidence suggests that one or more copies of the viral genome are attached to chromosomes of the cell (Weiss, Ephrussi, and Scaletta, 1968). Loss of the virus is accompanied by loss of transformed cell characters (Macpherson, 1965; Marin and Macpherson, 1969), so we assume that it is the virus which not only initiates the change but maintains it. This is also indicated by the fact that some cell changes may be specific for the transforming virus. It is well established, for example, that transformed cells carry virus-specific antigens, but there are also modifications in cell morphology which are typical for the strain of transforming virus (Temin, 1960).

FIG. 3.—A, Confluent layer of BHK cells in medium containing 10% serum. B, Confluent layer of BHK cells transformed by polyoma virus, and grown for the same time as (A) in medium containing 10% serum ($\times 955$) (from W. House and M. G. P. Stoker, *Journal of Cell Science*, 1966, 1, 169).

Certain changes, however, are common to cells transformed by many different tumour viruses, and perhaps also to tumour cells arising from the action of other carcinogens. Apart from transplantability these include the general relaxation of growth control detectable in culture, which we will now consider. Once again our own studies have been largely confined to the BHK 21 line of hamster fibroblasts and their derivatives transformed by polyoma virus. Model systems under intensive study in other laboratories include SV40 virus transformed 3T3 cells and Rous sarcoma virus transformed chick fibroblasts.

Growth Characteristics of Transformed Cells

Transformed cells grow in conditions which are restrictive for untransformed cells. Thus transformed cells are less sensitive to density-dependent inhibition, and will continue to multiply on a surface to form piled up layers many cells thick, while normal cells under similar conditions stop growing at a lower density varying with the cell type. Fig. 3 shows sections of untransformed BHK 21 cells and polyoma transformed cells derived from them, both grown under the same conditions. There are continuing cell divisions—that is, mitoses—deep in the layer of transformed cells.

Certain types of transformed cells, unlike normal cells, will also grow freely in suspension without contact with a rigid surface. This is a very useful attribute because it enables a small proportion of transformed cells to be detected in a large number of normal cells by observing the colonies which appear in suspension cultures, and it is the basis of an assay for transforming viruses (Macpherson and Montagnier, 1964).

Finally, transformed cells can grow for at least one or two divisions in concentrations of serum too low to permit growth of untransformed cells (Bürk, 1967). Even in complete absence of added serum transformed cells will continue to synthesize D.N.A. (Clarke *et al.*, 1970), though they subsequently degenerate without undergoing mitosis. This suggests that though transformed cells do not need serum factors which initiate D.N.A. synthesis, they still require other serum constituents for mitosis. Loss of requirement for a factor for the initiation of D.N.A. synthesis and the cell cycle could be enough to explain the loss of density dependence in virus-transformed cells. We have seen that dense cultures and also suspended cells grow poorly in conventional medium because in these states they have a high requirement for serum factor,

so transformed cells will have the biggest selective advantage over normal cells in just those conditions where the requirements for the factor are most exacting. This feature of transformed cells is shown diagrammatically in Fig. 4, which should be compared with Fig. 2.

Abortive and Stable Transformation

Before considering further the role of the transforming virus it is advisable to distinguish the hereditary from the physiological aspects of the transformation. The growth characteristics which I have mentioned refer to established lines of transformed cells, and the changes are stably inherited. When normal cells are exposed even to large doses of polyoma virus only a small proportion, up to about 5% of cells, show *stable* transformation. A few years ago, however, a second type of transformation was observed which affected a high proportion, and indeed sometimes all, of the cells exposed to virus (Stoker, 1968). This was called “abortive transformation” because the cells acquired the transformed characters for a few days only, and then reverted to the normal state. It was first noticed as a transient growth in suspension lasting for up to six divisions, but it is now studied more conveniently in surface cultures deprived of serum.

In addition to initiation of cell growth, cell movement begins as a result of infection and the cells develop the characteristic random orientation of transformed cultures. Since these characteristics are soon lost in most cells, and perpetuated in only the few which are stably transformed, we can conclude that transient alteration of cell physiology does not depend on the stable perpetuation of the transformed state. Further evidence comes from studies on a virus mutant called Tsa (Fried, 1965, 1970). The Tsa mutant can transform abortively but cannot give rise to stable transformants (Stoker and Dulbecco, 1969), so there is a viral gene required for stabilization, probably attachment of viral D.N.A. to a host chromosome. It is supposed that there must be at least one other “transforming” gene responsible for the changes, such as initiation of cell D.N.A. synthesis seen in abortive transformation, though no mutant of this supposed gene has yet been isolated. A plausible hypothesis would be that a viral gene product can transform the cell irrespective of whether the viral D.N.A. is free or attached to a host chromosome, but that attachment to a chromosome requiring other viral genes is needed for stable perpetuation, and this occurs rather rarely.

Initiation of Transformation

Since a high proportion of cells can be transformed abortively it is now possible to study the earliest cell changes without waiting for the emergence of the few stably transformed clones. Thus time-lapse cinematography of suspended BHK 21 cells exposed to polyoma virus shows that the first mitoses appear after about 30 hours, and that further mitoses continue at 12-hourly intervals, as in normal cells under experimental growth conditions (Stoker and Dulbecco, unpublished data). This suggests that the virus takes at least 18 hours to start the cycle off but that, once started, the cell does not have to begin again in succeeding cycles, perhaps because some necessary factor is then synthesized continuously.

So far I have stressed that polyoma virus and other tumour viruses allow growth by obviating the requirement for serum factor normally required for initiation of D.N.A. synthesis. But there are other physiological changes in cultured transformed cells, such as increased glycolysis, loss of contact, inhibition of movement, membrane changes, and the appearance of viral specific antigens. An outstanding problem concerns the role of the virus in affecting all these changes. The small D.N.A. viruses have a very limited genetic capacity, and

FIG. 4.—Schematic representation, as in Fig. 2, showing the lack of influence of serum and of cell morphology and contact state on D.N.A. synthesis in virus-transformed BHK cells.

it is probable that the information transcribed from the viral genome in transformed cells is sufficient for only two or three average-size proteins. To determine whether all the cell changes follow the expression of one viral gene, or more than one, will depend on the isolation and study of further viral mutants affecting transformation.

Another major problem concerns the relationship of these various manifestations of transformation to one another and to the initiation of a cell growth cycle. Is increased glycolysis, for example, or altered cell movement, related to initiation of D.N.A. synthesis, and if so is it a cause or a consequence? This may now be investigated by determining the time of appearance of the changes during abortive transformation in mass cultures to see whether they precede or succeed the growth initiation.

Conclusion

Studies on cultured fibroblasts have led us to postulate a form of growth regulation which involves a general extracellular factor, the action of which is affected by very local changes in the topography and perhaps the membrane state of individual cells. If this system operates in a whole organism such as the human body it is likely to be one of a large number of integrated sets of growth-regulation systems, and could be a relatively minor one. Nevertheless, the system deserves our attention, not just because it can be isolated for experimental investigation, but because it is by-passed in cells transformed with carcinogenic viruses. Since they have few genes these viruses cannot perform many operations and so cannot be doing much else even when starting a tumour in an animal. For that reason we may hope that the changes in growth observed in cultured cells may indeed be relevant to real cancer.

REFERENCES

- Abercrombie, M., and Heaysman, J. E. (1954). *Experimental Cell Research*, **6**, 293.
- Bullough, W. S., and Laurence, E. B. (1960). *Proceedings of the Royal Society Series B*, **151**, 517.
- Bullough, W. S., Laurence, E. B., Iversen, O. H., and Elgjo, K. (1967). *Nature*, **214**, 578.
- Bürk, R. R. (1967). In *Growth Regulating Substances for Animal Cells in Culture*, ed. V. Defendi and M. Stoker, p. 39. Philadelphia, Wistar Institute Press.
- Clarke, G. D., Stoker, M. G. P., Ludlow, A., and Thornton, M. (1970). In press.
- Dulbecco, R. (1970). In press.
- Dulbecco, R., Hartwell, L. H., and Vogt, M. (1965). *Proceedings of the National Academy of Sciences of the United States of America*, **53**, 403.
- Dulbecco, R., and Stoker, M. G. P. (1970). In press.
- Fried, M. (1965). *Virology*, **25**, 669.
- Fried, M. (1970). *Virology*, **40**, 605.
- Healey, G. M., and Parker, R. C. (1966). *Journal of Cell Biology*, **30**, 539.
- Holley, R. W., and Kiernan, J. A. (1968). *Proceedings of the National Academy of Sciences of the United States of America*, **60**, 300.
- Levine, M., Becker, Y., Boone, C. W., and Eagle, H. (1965). *Proceedings of the National Academy of Sciences of the United States of America*, **53**, 350.
- Macpherson, I. (1965). *Science*, **148**, 1731.
- Macpherson, I., and Montagnier, L. (1964). *Virology*, **23**, 291.
- Macpherson, I., and Stoker, M. (1962). *Virology*, **16**, 147.
- Marin, G., and Macpherson, I. (1969). *Journal of Virology*, **3**, 146.
- Martin, L., and Finn, C. A. (1968). *Journal of Endocrinology*, **41**, 363.
- Stoker, M., and Dulbecco, R. (1969). *Nature*, **223**, 397.
- Stoker, M. (1968). *Nature*, **218**, 234.
- Stoker, M., O'Neill, C., Berryman, S., and Waxman, V. (1968). *International Journal of Cancer*, **3**, 683.
- Temin, H. M. (1960). *Virology*, **10**, 182.
- Temin, H. M. (1967). *Journal of Cellular Physiology*, **69**, 377.
- Temin, H. M. (1968). *International Journal of Cancer*, **3**, 771.
- Todaro, G. J., and Green, H. (1963). *Journal of Cell Biology*, **17**, 299.
- Todaro, G. J., Lazar, G. K., and Green, H. (1965). *Journal of Cellular and Comparative Physiology*, **66**, 325.
- Todaro, G., Matsuya, Y., Bloom, S., Robbins, A., and Green, H. (1967). In *Growth Regulating Substances for Animal Cells in Culture*, ed. V. Defendi and M. G. P. Stoker, p. 87. Philadelphia, Wistar Institute Press.
- Weil, R., Michel, M. R., and Ruschmann, G. K. (1965). *Proceedings of the National Academy of Sciences of the United States of America*, **53**, 1468.
- Weiss, M. C., Ephrussi, B., and Scaletta, L. J. (1968). *Proceedings of the National Academy of Sciences of the United States of America*, **59**, 1132.
- Winocour, E., Kaye, A. M., and Stoller, V. (1965). *Virology*, **25**, 156.

Fibrin Degradation Products in Pre-eclamptic Toxaemia and Eclampsia

A. H. HENDERSON,* M.B., M.R.C.P. ; D. J. PUGSLEY,† M.B., M.R.C.P. ; D. P. THOMAS,‡ M.D., B.SC., D.PHIL.

British Medical Journal, 1970, **3**, 545-547

Summary: Serum levels of fibrinogen/fibrin degradation products, measured in African women, were significantly higher in pre-eclamptic toxæmia than in normal pregnancy, and were significantly higher with eclampsia than with toxæmia. These findings are in accord with the hypothesis that eclampsia and toxæmia are associated with disseminated intravascular coagulation, which may be responsible for certain clinical manifestations of these conditions.

Introduction

It has been suggested that disseminated intravascular coagulation is responsible for many of the manifestations of pre-eclamptic toxæmia and eclampsia (McKay, Merrill, Weiner, Hertig, and Read, 1953; McKay and Corey, 1964). Disseminated intravascular coagulation is characterized by the consumption of platelets, fibrinogen, and blood-clotting factors V and VIII, leading to a decrease in their circulating levels if

the process is sufficiently acute. There is also an increase in the level of circulating fibrinogen/fibrin degradation products (F.D.P.), produced as a result of secondary, or compensatory, fibrinolysis. Obstructive microangiopathy may follow the formation of intravascular thrombi, with subsequent deposition of fibrin. Red cell fragmentation may also occur, and this has been attributed to mechanical damage sustained as red cells pass through a fibrin mesh in still patent vessels (McKay and Shapiro, 1958; Brain, Dacie, and Hourihane, 1962; McKay, 1964).

The clinical, histological, and haematological changes of eclampsia, and to a lesser extent of toxæmia, show many similarities to other clinical features attributed to disseminated intravascular coagulation (Taub, Rodríguez-Erdmann, and Dameshek, 1964). Thus there are the signs of renal disease which may progress to acute renal failure; convulsions which may occur even in the absence of severe hypertension; and fibrin-like material which has been demonstrated in the endothelium of small blood vessels from many organs in eclampsia and in the kidney in toxæmia (Govan, 1961; Vassalli, Morris, and McCluskey, 1963; McKay, 1964). Red cell fragmentation and thrombocytopenia have been reported in eclampsia, with increased platelet stickiness in toxæmia

* Senior Registrar in Medicine, Royal Devon and Exeter Hospital, Exeter.

† Registrar in Medicine, Makerere University College, Kampala, Uganda.

‡ Associate Professor of Medicine, Tufts University School of Medicine; Physician-in-charge, Vascular Laboratory, Lemuel Shattuck Hospital, Boston, Massachusetts.