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PRINCIPLES OF CELL MOTILITY: THE DIRECTION OF CELL MOVEMENT AND CANCER INVASION

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INVESTIGATIONS of cell motility are generally carried out with cells attached to glass surfaces, and, in many cases, the influence which the glass itself may have on patterns of cell movement and behaviour is not fully taken into account. The use of unconventional transparent materials serves to emphasize the importance of the part played by the substrate in cell motility. Cellulose acetate has been selected from a wide range of available materials as being of particular value in experiments of this type.

Mammalian cells in culture do not readily adhere to a surface of cellulose acetate, but if this surface is metallized *in vacuo*, cultured cells will adhere to it and spread. By varying the amount of metal deposited it is possible to produce a range of surfaces allowing different degrees of cell adhesion.

The investigation of cell behaviour on various surfaces prepared in this way has led me to draw a number of general conclusions about the principles which govern the direction of cell movement, and the mechanism of cell motility itself.

In all the experiments to be described the cells used were mouse fibroblasts (Earle's *L* strain), and these were cultured in Eagle's medium[1], with 8 per cent calf serum. A culture chamber of new design was used and this will be described elsewhere. Cover glasses may be coated with an acetate film by immersing them in a 0·5 per cent solution of cellulose acetate (acetyl value 54–56 per cent) in 1,4-dioxan. These are drained in an atmosphere saturated with dioxan, and dried under vacuum to avoid clouding by atmospheric humidity. The dried films are then heated to 280° C for 10 sec, a procedure which melts the acetate layer, fixing it firmly to the glass and sterilizing it at the same time. The surface of the film must be protected from physical and chemical contamination. Purity of materials is essential.

Metallic palladium is deposited on the acetate surface in a vacuum coating unit of the type used for shadow casting in electron microscopy. A measured length of fine palladium wire is placed on a cleaned molybdenum strip and heated to white heat at 10^{-4} mm mercury. The vaporized palladium is deposited on the acetate film by placing the cover glass above the molybdenum strip at a height which can be varied to control the amount of metal deposited. The minimum quantity of palladium necessary to allow full cell adherence to the film gives a very fine deposit which cannot be detected by eye. Several other metals have been found to be equally effective in this respect, but palladium has proved to be the most convenient and there have been no indications of it being responsible for any toxicity.

In order to make a gradient of metal deposition and therefore a surface offering progressively increasing cell adhesion, a number of techniques have been used. The simplest of these is to place a short length of stainless steel rod, 0·5 mm in diameter, in contact with the acetate film. The film is then shadowed by vaporizing 2 mg of fine palladium wire, rolled into a small pellet, at a distance

of 10 cm. This makes a relatively heavy deposition of metal which clearly outlines the intervening rod. Owing to the scattering of metal particles, however, a much finer deposit of palladium extends beyond the visibly shadowed area, and tapers off in the narrowing angle under the curved surface of the rod. A gradient of metal deposition is therefore produced. The advantage of this method is that the upper limit of the gradient is clearly defined by the visible edge of the densely shadowed area.

Cell Movement on a Gradient of Adhesion

Fig. 1 shows a number of cells photographed shortly after attachment, lying on an adhesion gradient prepared in this way. The full metal deposit is visible as a darker area in the upper part of the photograph, and the gradient extends from the bottom of the photograph to the edge of this darker area. Fig. 2 shows the same field after an interval of 8 h, and Fig. 3 shows the tracks of all cells during this period, plotted from a series of intermediate photographs.

It will be seen that the cells on the gradient move in the direction of increasing adhesion to the substrate, and their uniformity of movement contrasts with the apparently random movement of cells on the evenly metallized cellulose acetate, or on glass. As the cells crowd together at the upper end of the gradient, progressively increasing cell-to-cell contacts interfere with the movement of individual cells. Nevertheless the cell migration continues in the same direction, and Fig. 4 shows the same field again after 48 h. At this stage the free edge of the cell sheet is retracting, whereas under more usual conditions a free edge of this type would advance.

Not only is cell movement on the gradient directional, but also the cell tracks are longer and indicate more efficient motility. This is particularly evident in less-crowded cultures where there is more room for individual cell movement, and in a number of experiments of this type the average length of cell track on the gradient was four to five times as long as that on the evenly metallized surface. The speed of cell movement on the evenly metallized surface is of the same order as it is on glass under similar conditions.

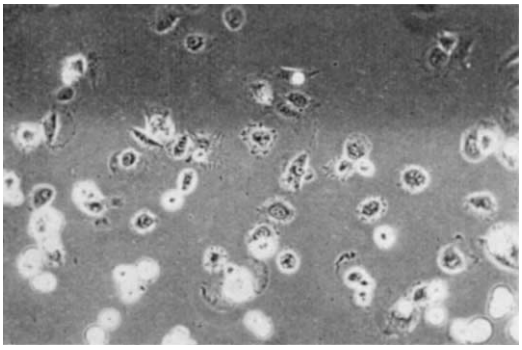

Fig. 1. Newly attached cells on an adhesion gradient. Substrate adhesion increases from the bottom of the photograph to the edge of the darker area. (× 125.) (All photomicrographs in this article were taken by phase contrast illumination.)

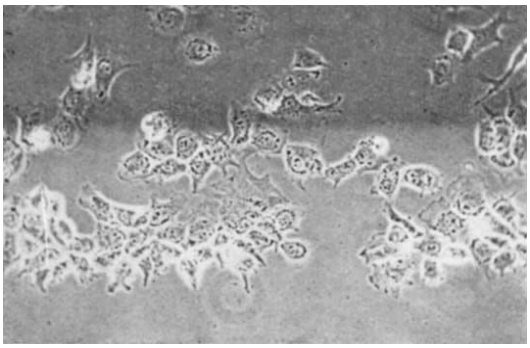

Fig. 2. The same field as Fig. 1 after an interval of 8 h. The cells have moved up the adhesion gradient towards the darker area at the top. (× 125)

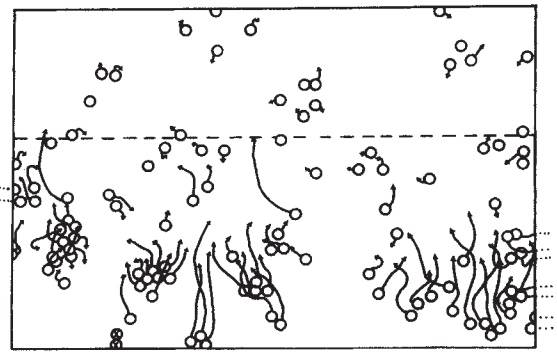

Fig. 3. The movement of individual cells on an adhesion gradient. The circles represent the original positions of the cell nuclei in Fig. 1, and the lines trace the paths followed by these nuclei to their positions in Fig. 2 8 h later. The two nuclei marked with crosses represent cells which became detached. (× 125)

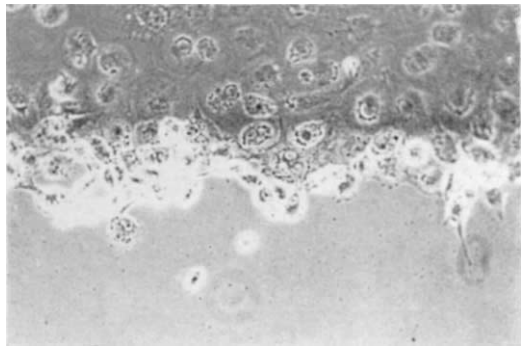

Fig. 4. The same field as in Fig. 1 after 48 h. The cells have continued to move up the adhesion gradient in spite of the crowding which results. The two isolated cells are late-comers which were outside the original field shown in Fig. 1. (× 125)

It seems probable that movement towards surfaces offering greater adhesion is a general phenomenon applicable to all metazoan cells which are dependent on contact with a surface for their motility, and I believe that this is the essential principle which directs and controls all cell movements of this type. It is generally appreciated that cell motility and cell adhesion are interrelated, but the directness and simplicity of this relationship have not previously been demonstrated by experiment.

Gustafson and Wolpert[2] suggested on theoretical grounds that cells would tend to move up a gradient of increasing substrate adhesion, but the mechanism they proposed, involving competition between randomly extended pseudopodia, would not predict the immediately directional movement of individual cells which in fact occurs. I suggest that this phenomenon should be called 'haptotaxis' (Greek: haptein, to fasten; taxis, arrangement), conveying the idea that the movement of a cell is controlled by the relative strengths of its peripheral adhesions, and that movements directed in this way, together with the influence of patterns of adhesion on cell shape, are responsible for the arrangement of cells into complex and ordered tissues.

On this basis, the cell migrations involved in morphogenesis, inflammation, wound healing, tumour invasion and indeed all tissue cell movements, are considered to be the result of haptotactic responses by the cells involved, and correspondingly the relative stability of cells in differentiated adult tissues is due essentially to the lack of adhesion differentials on which effective cell movement depends. In discussing the possible mechanisms which

might control cell movement, Weiss[3] pointed out that any such mechanism must involve selective conduction or selective fixation. His third principle of selective elimination is not strictly concerned with cell motility. It is interesting to note that the idea of haptotaxis effectively combines both these principles of cell movement.

Contact Inhibition

The concept of contact inhibition[4] is valuable in providing a primary explanation for the differences in behaviour between normal and malignant cells. Cells which inhibit movement in each other on contact, and therefore tend to form monolayers in culture, are said to show contact inhibition; whereas those which move over each other and pile up into multiple layers are said to show loss of contact inhibition. To a limited extent this distinction divides normal from malignant cells, and a change in the pattern of cell growth towards loss of contact inhibition serves to indicate the transformation of cells, for example by oncogenic viruses.

However, by varying the amount of palladium deposited on a cellulose acetate film and producing surfaces which allow different degrees of cell adhesion, it is possible to change completely the pattern of growth of the same type of cell, without otherwise altering the cultural conditions.

The cells in Fig. 5 are arranged in a monolayer and are demonstrating contact inhibition. These cells are attached to a cover glass which has been coated with acetate and exposed to vaporized palladium. Fig. 6 shows cells from the same culture as they appear on a different part of the cover glass where it has been masked to reduce the amount of palladium deposited. Here the cells are clumped together and would be said to demonstrate loss of contact inhibition. An intermediate stage in which cells show a tendency to monolayer, but with many cells overlapping, can be found in a narrow region between the two areas showing these strikingly different patterns of growth. This finding indicates that contact inhibition, or lack of it, cannot be regarded as an attribute of the cell itself, but describes its relationship to other cells in the presence of a particular alternative substrate.

The phenomenon of contact inhibition can be explained on the basis of haptotactic movement. Cells showing contact inhibition on glass are demonstrating that they can adhere more strongly to this material than they can to each other. Cell movement will therefore be towards any free glass surface so that the monolayer is preserved. Cells showing loss of contact inhibition on glass can adhere more strongly to each other, and will tend to leave the glass surface and pile up in multiple layers. This type of explanation is similar to one of several possibilities considered by Abercrombie and Ambrose[5], but Abercrombie[6] has since suggested that the explanation least open to

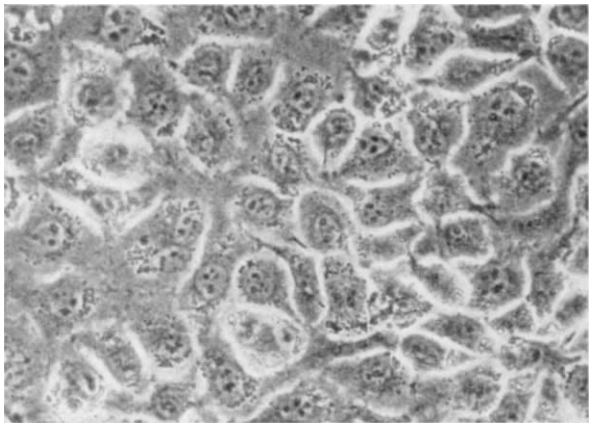

Fig. 5. 'L' cells showing contact inhibition on metallized cellulose acetate. (× 325)

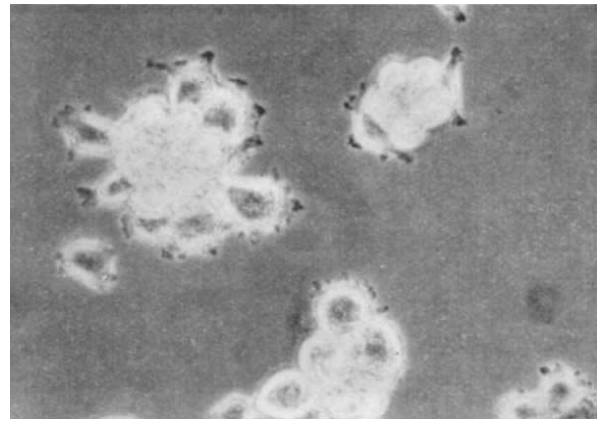

Fig. 6. 'L' cells showing loss of contact inhibition on very lightly metallized cellulose acetate. These cells were growing on a different area of the same cover glass as those shown in Fig. 5. (× 325)

objection is that adhesion between cells inhibits the ruffled membrane, as argued by Curtis[7,8]. In putting the opposite case it should be made clear that it is a relative lack of adhesion to the new substrate which is suggested as the operative mechanism, and this need not mean a low degree of adhesion in absolute terms. It is also important to emphasize that in discussing the adhesion of cells as it affects their motile behaviour, I am referring to the adhesion which occurs immediately on contact. The adhesion between cells which may develop on more prolonged contact is not at present being considered. The argument used by Abercrombie[9] against this type of explanation for contact inhibition is that it fails to account for the paralysis of the ruffling movements of the cell. In a later paper concerned with the mechanism of cell motility, I hope to show that this objection can be overcome.

The arbitrary choice of glass as the surface on which most cells in culture are investigated makes the description of a cell in terms of contact inhibition equally arbitrary, though under standardized conditions not without practical value. Unfortunately glass exists in many forms, and its surface properties are readily changed by washing procedures and by substances in the culture medium itself.

Contact Guidance

The principle of contact guidance, elaborated by Weiss[10], can also be explained on the basis of cell movement along a path of preferential adhesion. Although this mechanism is to a large extent implied by Weiss, he argues against the acceptance of any simple explanation in these terms. Orientated structures are likely to present a correspondingly orientated pattern of differing adhesion. This may be so even if the components in the orientated structure are all of the same material, since curvatures of the surface will affect the relative densities of the surface elements which may be concerned with adhesion.

Very precise contact guidance can be achieved by cutting narrow channels in a thin film of cellulose acetate so that the underlying glass is exposed, thereby providing a path offering far greater adherence for cells than the surrounding acetate (Fig. 7). Weiss has suggested that the mechanism of contact guidance may involve micro-orientation of the guiding surface, and has considered how this orientation could be transferred to the molecules of the cell surface in contact with it, and hence to the cell as a whole. It seems unlikely that there should be an appropriately orientated microstructure on a glass surface which has been exposed by removing a narrow strip of cellulose acetate, although such a microstructure could conceivably be imposed by the cutting instrument used to remove it.

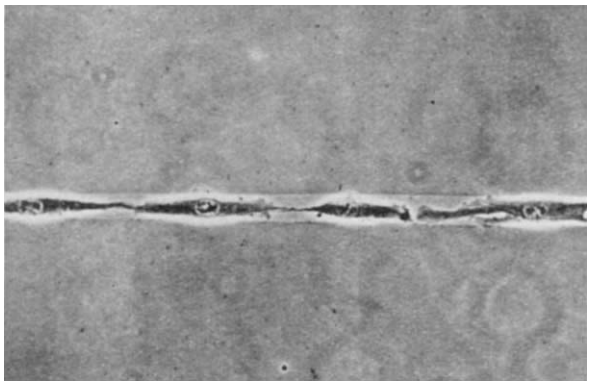

Fig. 7. Contact guidance along a channel cut through a film of cellulose acetate on glass. (× 225)

Contact guidance can also be demonstrated on cellulose acetate when the path of preferential adhesion is made by overlaying with vaporized palladium rather than cutting down to underlying glass.

Such a path can be made by masking the acetate surface and restricting the deposition of metal to a strip of any desired width. Fig. 8 shows cells demonstrating contact guidance along a path made in this way. There is no reason why a metallized strip should have an orientated microstructure, and the simple interpretation involving differential adhesion alone seems adequate as a primary explanation for contact guidance phenomena.

Chemotaxis

There are probably many thousands of substances which can evoke chemotactic responses *in vivo*, but the number which demonstrate this effect under strictly *in vitro* conditions is remarkably small[11]. The explanation for this may be that the chemotactic substance is acting indirectly by damaging or otherwise altering the surfaces of cells and non-cellular structures in its neighbourhood. This surface change enables the normally non-adherent leucocyte, for example, to adhere. Since the surface change will be greatest in closest proximity to the chemotactic agent, a gradient of surface adhesiveness will be formed to guide the leucocyte to the appropriate area. Such a gradient may be reinforced by the action of substances released by the damaged cells, and it is probable that these substances rather than the chemotactic agent itself are mainly responsible for the adhesion gradient produced. Chemotaxis is difficult to demonstrate *in vitro* because artificial substrates are less susceptible to these surface changes. A direct effect on the leucocyte is also possible since the leucocyte itself could suffer surface changes which would make it more adhesive on the side nearest to the chemotactic stimulus. Failure to demonstrate *in vitro* responses to the majority of agents which are chemotactic *in vivo* suggests that this direct effect may be relatively unimportant. The suggestion that chemotaxis is a special case of haptotaxis applies equally well to negative chemotaxis, but in applying this principle there is no need to consider it in negative terms.

Cancer Invasion

If malignant invasion is due to the operation of haptotaxis, the implication would be that cancer cells can form stronger adhesions to surrounding normal cells than they can to each other.

It was originally shown by Coman[12] that cancer cells have a reduced mutual adhesiveness, and it has also been found that the net negative charge on the surface of a virus-transformed cell is higher than the surface charge on a homologous normal cell[13]. It seems likely that these two findings are closely related, and on theoretical grounds it might be expected that a cancer cell would adhere more firmly to a normal cell than to another cancer cell because the repulsion due to the combined surface charges would be less.

To obtain absolute measurements of the adhesion between two cells of individually known type would be difficult, but if haptotaxis is accepted as a principle of cell movement, then the direction of cell movement itself can be used to give an indication of the relative adhesiveness of two surfaces for a particular cell.

Abercrombie, Heaysman and Karthauser[14] made the important observation that confronted outgrowths from mouse sarcoma and chick embryo tissue showed more cell overlaps than occurred in a corresponding experiment with two cultures of chick tissue. This involves a comparison of normal and malignant cells from unrelated species, and the experiment is further complicated by the presence of a third surface in the form of a plasma coagulum. Although it points in the right direction, an experiment of this complexity would be difficult to interpret with confidence in terms of relative surface adhesion.

The value of virus transformation in culture is that it makes possible the comparison of normal cells with cells which are as nearly as possible their malignant counterparts. Vogt and Rubin[15] observed that chick fibroblasts transformed by Rous sarcoma virus form multi-layered clumps on glass, but migrate readily over the sheet of untransformed cells. This suggests that the transformed cells are more adherent to themselves than to glass and more adherent to corresponding normal cells than to each other. Even so, it would simplify the interpretation of this experiment to eliminate any possible interference by the glass so that the comparison can be restricted to the cell surfaces only. For this reason some interesting experiments by Stoker[16] are particularly relevant. He showed that whereas hamster cells transformed by polyoma virus formed distinct heaped-up colonies on glass, these same cells did not produce colonies when seeded onto pre-existing monolayers of normal hamster cells. Although he found evidence that the rate of division of the transformed cells was reduced under these conditions, their failure to form multi-layered colonies could also indicate that they were spreading over the pre-existing monolayer and, therefore, demonstrating a stronger attachment to normal cells than to each other. Stoker's experiments were not, of course, designed to test this hypothesis, and his own interpretation involves an entirely different theory in relation to the mechanism of contact inhibition. Nevertheless, this type of experiment closely parallels the experiment previously described in which cells were shown to clump or spread out according to the adhesiveness of the underlying surface.

Although these experiments with transformed cells point to the conclusion that malignant cells can adhere more strongly to their normal counterparts than to each

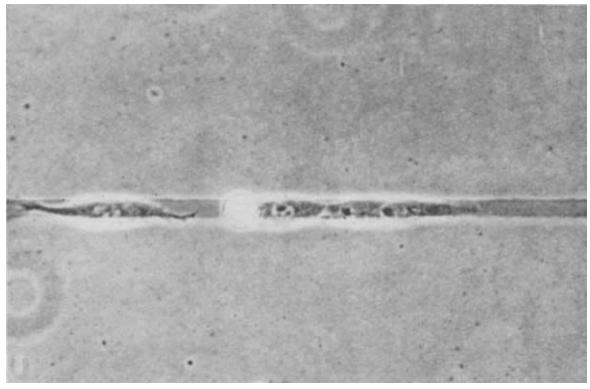

Fig. 8. Contact guidance on a cellulose acetate film coated in a narrow strip with evaporated palladium. (× 225)

other, it is clear that further experiments are required, and these need to be specifically designed to make interpretation in these terms as direct as possible. In addition, it is necessary to establish haptotaxis more firmly as the overriding principle of cell movement. In order to do this it is essential to relate it to the underlying mechanism of cell motility, and this will be attempted in a subsequent paper.

On the basis of haptotactic cell movements, many aspects of the natural history of malignant disease can be interpreted. In local invasion, the peripheral tumour cells move away from the tumour mass into the surrounding tissue in response to the adhesion differential, and are followed immediately by other cells moving under the same influence. In this way, a column of cells tends to be formed and the process of malignant invasion becomes more consistent in direction and consequently more efficient than if individual cells were cut off from the tumour mass so that they were no longer subject to the directional cue provided by the presence of normal cells in front and malignant cells behind.

The pattern of invasion is affected by tissue architecture, not simply on the basis of the physical structures encountered, but also in accordance with the degree of adherence that the cellular and non-cellular components of the structure offer to the invading cells. Similarly the pattern of metastasis for a particular type of tumour is partly determined by anatomical considerations, and partly by the adhesiveness of the endothelial cells lining blood vessels and lymphatics in different tissues for the particular type of malignant cell. The surface properties of these endothelial cells would be expected to vary according to the differing physiological environments to which they are subjected in different types of tissue.

The invasiveness of a particular tumour cannot be regarded as a characteristic solely of the tumour itself, since the nature of the surrounding normal cells plays a part in determining the degree to which tumour to normal cell adhesion exceeds mutual tumour cell adhesion. For this reason, a tumour metastasis in a different tissue may show a greater or a lesser degree of invasiveness in its new site when compared with the primary tumour, and it would be predicted that some tumours which are non-invasive and regarded as 'benign' in their primary sites could become 'malignant' if artificially transferred elsewhere.

It is important to bear in mind that the cells involved in malignant invasion are not moving in a stationary field. All tissue cells are potentially motile, and normal cells may move in relation to malignant cells for precisely the same reasons as malignant cells move in relation to normal cells. The movement of fibrocytes, for example, in contact with some types of neoplastic cells can result in the effective walling off and containment of a tumour within a fibrous capsule, and the penetration of fibrocytes into the substance of a carcinoma can convert it into a scirrhous type of tumour. The infiltration of some tumours by lymphocytes is also a clear case of the 'invasion' of cancerous tissue by normal cells. It may be more rewarding to consider such phenomena with reference to a defined concept such as haptotaxis than to describe them in non-committal terms of 'reaction' to the tumour. Only a brief indication of the application of such a concept has been attempted here.

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A SEX-DEPENDENT FACTOR IN APLASTIC ANAEMIA?

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APLASTIC anaemia is characterized by the deficient production of peripheral blood cells and is nearly always attended, and presumably caused, by severe bone marrow hypoplasia. The fundamental cause of the condition is usually unknown, and although spontaneous remissions do occur, they are not common, and treatment has usually been conspicuously unsuccessful. Animal experiments in bone marrow transplantation indicated a possible new approach to the management of this disease by the grafting of healthy bone marrow cells: allogeneic marrow infusions are attended by difficulties and hazards which are avoided by the use of syngeneic donors, and on the rare occasions when a patient with aplastic anaemia has a normal identical twin it is possible to duplicate this favourable circumstance clinically. Seven such cases have been reported (Table 1).

In three of the four successful cases recovery was rapid and complete (Cases 2, 4 and 5), while in the fourth (Case 6) the response was slow and incomplete following the first marrow infusion, but a second infusion was more satisfactory although some thrombocytopenia persists[8]. Of the unsuccessful cases, the first (Case 1) has since undergone a slow and partial recovery while under treatment with steroids and testosterone (severe thrombocytopenia persists)[9], Case 3 was not significantly benefited, and Case 7 died one month later. In each of the four successful cases the author of the report was convinced that the treatment had been responsible. It is of course necessary to consider the possibility that the recoveries were spontaneous and coincided with the treatment purely by chance. This seems highly improbable: the spontaneous recovery rate is low, particularly when the disease has been present for a considerable time, as it had in Cases 4, 5 and 6 particularly. If it is accepted that the treatment was responsible for the recovery of the four successful cases, some attempt must be made to account for its failure in the other three. There appears to be no correlation between the result of the treatment and the cause of the aplasia (in so far as it is known), the age of the subject, or the amount of marrow injected: it is striking, however, that all the female cases recovered whereas all the male cases did not. To calculate the probability of this result occurring purely by chance, where the true reason is the operation of a factor not dependent on sex, it is necessary to make some assumption concerning the number of cases expected to recover. Table 2 shows the calculated probability for a selection of overall success rates. The highest probability is given by assuming that four recoveries will occur in any seven cases treated (the observed success rate) and on this assumption the probability of the observed distribution of successes and failures between males and females