A Haematologist's Approach to Modern Grafting Problems*

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The position of haematologists regarding the graft problem can be summarized in the words of Ernest Renan: "Nothing great is accomplished without the Chimaera" (*L'avenir de la Science*). In fact, haematologists are directing their efforts towards the creation of chimaerae. If they succeed the application of their results will extend beyond the realms of haematology. To-day there are indeed three major applications of haemopoietic cell grafts: (1) As replacement treatment of myeloid insufficiency (myeloid tissue includes the erythrocytic, granulocytic, and megakaryocytic-thrombocytic lines); (2) for the treatment of some neoplastic affections; (3) for the establishment of specific immune tolerance associated with chimerism so as to make tissue and organ transplants possible.

We propose to devote the first part of this lecture to an exposition of the three types of application; the second part will deal with various problems and practical difficulties encountered in grafting haemopoietic cells.

Replacement Treatment of Myeloid Insufficiencies by Grafting Haemopoietic Cells

This treatment gives results that vary widely according to the aetiological background. Isogeneic¹ grafts of haemopoietic cells cause no difficulty. In principle they may be used in the treatment of all varieties of myeloid insufficiency. It has been demonstrated in experiments with mice that it is possible in this way to achieve rapid restoration of bone-marrow rendered aplastic by chemical antimitotics (Tran Ba Loc *et al.*, 1958) or by total α -irradiation (Lorenz *et al.*, 1951); the treatment reduces the mortality of aplasia in animals (Van Bekkum *et al.*, 1956) and in man (Mathé, 1962) regardless of the irradiation dosage. The only evidence that such isogeneic transfusions do lead to myeloid restoration is the statistical comparison of survival rates in treated subjects and in controls identically irradiated but not treated (Mathé, 1962). It is probable, moreover, that grafts of isogeneic elements are only transient, and that the marrow more or less rapidly returns to its autologous populations. In a human case given 800 rads γ -irradiation and treated by transfusion of isogeneic marrow which was followed by a rapid restoration, this return of autologous cells was suggested by the occurrence of chromosomal anomalies manifestly connected with the irradiation and seen in numerous

mitoses a few weeks after restoration (Papiernik-Berkbauer *et al.*, 1963).

In mice, a genetically determined congenital anaemia has been successfully treated by irradiation and co-isogeneic marrow graft (Russell *et al.*, 1959).² A similar experiment in man, which would be of greater importance, is lacking.

In animals, a graft of allogeneic³ haematopoietic cells has been demonstrated to be possible only in two circumstances: (a) in adults affected by myelolymphoid aplasia due to total body α -irradiation at a lethal dosage and sometimes at a sublethal dosage (Uphoff, 1963); (b) in neonates (Amiel and Mathé, 1960).

Although in man many attempts have been made to transfuse allogeneic bone-marrow in cases of aplasia or myeloid hypoplasia of various origins, with variable success, few cases have been analysed on strict lines to determine whether the graft really took; these were cases of aplasia due to chemical antimitotics or total body α - or γ -irradiation. Mention can be made of two positive results in patients with myeloid lymphoid aplasia, in one case caused by *p*-bis-(2-chlorethyl-amino)-phenyl-butyric acid (Beilby *et al.*, 1960), and in the other due to thio-triethylene-phosphoramidate (Bridges *et al.*, 1960).

Personal Observations

We will confine ourselves to an analysis of our own experiences. This concerns 23 subjects who can be divided into three groups. The first group includes eight patients with leukaemia submitted in the terminal phase to total body α -irradiation with the aid of a conventional radiotherapy apparatus or with the betatron, at a relatively low, fractionated dosage (200-400 rads); in none of these cases did transfusion of allogeneic marrow result in a successful graft (Mathé, 1959).

The second group (Mathé *et al.*, 1959b) concerns five subjects accidentally irradiated at the Nuclear Centre of Vinca (Yugoslavia) with a mixture of γ -rays and neutrons, at dosages for which a variety of estimates have been given but which we judge, after studying the physical, clinical, haematological, and histological data, as exceeding LD₇₅ for four of these patients, and exceeding LD₅₀ for the fifth (Mathé, Amiel, and Schwarzenberg, 1963a). These subjects had been given a transfusion of bone-marrow at different dates, the time being determined in each case by the presumed failure of symptomatic treatment. The transfusions were followed by myeloid restoration, which we attributed to the establishment of a graft of transfused elements for two reasons: (a) Although the subjects were

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¹ An isogeneic graft is a graft between individuals when the grafted cells have the same genes as the cells of the recipient. In man this is possible only when donor and recipient are identical—that is, monozygotic—twins. In experiments with animals which have been bred by brother-sister matings for many generations there is a similar degree of genetic similarity as between identical twins, and any graft from one such animal to another is an isogeneic graft. When cells from an individual are grafted back into the same individual the graft is called autologous.

² When all the genes are the same except at one locus, then the cells or individuals are coisogenic with each other. In the experiment of Russell *et al.* (1959) the congenitally anaemic mice were *WW^v* and the grafted cells contained the normal alleles *ww*.

³ An allogeneic graft is a graft of cells obtained from a donor of the same species as the recipient but genetically different from the recipient's cells.

irradiated at the same time, and in four cases at the same dosage, they showed myeloid restoration at different times, strictly dependent on the respective dates of marrow transfusion; (b) by virtue of differences in erythrocytic phenotype between donors and recipients, it was possible to calculate the quantity of red cells produced by each graft. For three weeks this was equivalent to the entire new production of erythrocytes. This quantity of blood attributable to the grafts increased only during three weeks, and then diminished, indicating the return of autologous production. The evidence suggested a transient graft, the therapeutic value of which is demonstrated by the fact that only one subject died, although four had received a dose exceeding $LD_{7.5}$ (the deceased patient had shown excellent myeloid restoration, but succumbed to intestinal lesions). None of these subjects developed a clinical secondary syndrome, probably because of the transient nature of the graft.

The third group includes 10 leukaemic patients deliberately submitted to total body γ -irradiation (Cobalt-60) at a dosage of about 800 rads and treated by transfusion of allogeneic marrow (Mathé *et al.*, 1959a; Mathé *et al.*, 1960b; Mathé *et al.*, 1963b; Mathé *et al.*, 1964a; Mathé *et al.*, 1964b). The results can be described as follows: (a) in three patients (Mathé *et al.*, 1959a; Mathé *et al.*, 1960b) the attempt at transplantation failed—it was not followed by restoration; two died of aplasia due to γ -irradiation; in the third case (Mathé *et al.*, 1964b) restoration was subsequently affected after transfusion of isogeneic bone-marrow. (b) All other patients showed myeloid restoration after allogeneic marrow transfusion, but the course varied because of different aspects of the secondary syndrome complicating the graft (Mathé *et al.*, 1959a; Mathé *et al.*, 1964b). In two the secondary syndrome was exceedingly severe and early, occurring at the onset of myeloid restoration before there was time to repopulate the blood (Mathé *et al.*, 1964b). In three patients the secondary syndrome occurred after myeloid restoration had occurred and had repopulated the blood; two of these died in spite of correcting the neutropenia and thrombocytopenia (Mathé *et al.*, 1960b); in the third, however, the secondary syndrome was controlled although it was very severe and protracted over a period of two months (Mathé *et al.*, 1963b, 1964a). In two patients the secondary syndrome occurred one month after myeloid restoration; it lasted about one month and was controlled without difficulty (Mathé *et al.*, 1959a).

In subjects in whom the marrow was restored various proofs of grafting were found: (a) production of erythrocytes showing the donor's antigenic characteristics; (b) production of female leucocytes in male recipients who were transfused with marrow from female donors.

In subjects who survived the secondary syndrome the graft lasted variable periods of time. It survived three months in two patients whose secondary syndrome was late and of minor intensity (Mathé *et al.*, 1959a); another graft has lasted 16 months in a patient whose early and severe secondary syndrome could be controlled (Mathé *et al.*, 1963b, 1964a).

These are our personal observations on the possibility of using bone-marrow grafts in the replacement treatment of myeloid insufficiency.

Treatment of Some Neoplastic Affections

The use of isogeneic bone-marrow grafts (Thomas *et al.*, 1959) or even autologous grafts (Kurnick *et al.*, 1959) as a means of protection against the risks of total body x - or γ -irradiation given as a therapeutic measure for certain disseminated neoplasias is of no great interest because there exists no cancer with cells sufficiently radiosensitive as to be wholly destroyed by total body irradiation at the maximum dosage which can be tolerated with the support of such grafts. It is possible, however, to obtain remissions in some cases of acute

leukaemia. Isogeneic or autologous bone-marrow grafts can also be used to protect those cases receiving chemotherapy in large doses (McFarland *et al.*, 1959).

A more interesting application in this field of study is the use of grafts of allogeneic³ haemopoietic cells as a means of adoptive immunotherapy, such as when immunologically competent cells of a bone-marrow graft react against leukaemic cells or antigens of a recipient. Let there be a subject (A), suffering from leukaemia caused by (α) virus and total body x -irradiation is administered at the maximum dosage tolerated (about 1,000 rads). On the one hand, the number of leukaemic cells is reduced, particularly if they are radiosensitive. On the other hand, the subject is conditioned for a transplantation of haemopoietic cells from donor (B). This transplantation is followed by a reaction of the immunologically competent cells of (B) against the normal (A) antigens (which determines the secondary syndrome) but also against the leukaemic cells and against a possible virus (α). In this way the grafted cells can be utilized as antileukaemic agents.

This reaction of immunologically competent graft cells against leukaemic cells has been demonstrated in experimental leukaemia (Barnes and Loutit, 1957; Mathé and Bernard, 1958, 1959; Mathé *et al.*, 1960a; Mathé *et al.*, 1962; De Vries and Vos, 1958), and was recently confirmed in a case of solid tumour (Woodruff and Symes, 1962). Our experimental studies concerned transplantable leukaemia, leukaemia L 1210, and a spontaneous, AkR leukaemia. We found that the reaction could postpone the animal's death from leukaemia and even eradicate the latter, but the intensity of the reaction was parallel to that of the reaction of the haemopoietic graft against the normal antigens. Animals in which the leukaemia was eradicated often died as a result of the secondary syndrome.

To demonstrate the reaction of a haemopoietic graft against a possible oncogenic virus, we have compared the effect on survival in two groups of mice suffering from Charlotte Friend's viral leukaemia (Mathé, Amiel, and Friend, 1962). Those in the first group were given total body x -irradiation and then restored by bone-marrow from normal isogeneic donors. Those in the second group were treated with marrow from isogeneic donors vaccinated with formalin-fixed virus. The second group showed a significantly higher survival rate.

Our results in man, although not sufficiently numerous to confirm definitely our line of thought, appear to be encouraging so far. Of three patients who survived the secondary syndrome (Mathé *et al.*, 1959a; Mathé, Amiel, and Schwarzenberg, 1963; Mathé *et al.*, 1963c, 1964a), one had a 26-months history of progressive leukaemia, in whom chemotherapy accomplished three remissions lasting one year, five months, and three weeks respectively. A remission lasting one year was obtained after bone-marrow grafting. The second patient had a 13-months history of progressive leukaemia; one remission of eight months and one of three weeks was obtained, which was replaced by a rapidly progressive relapse at the time when the therapeutic effort was made and a remission of nine months was obtained. The third patient, after 20 months' progression, began a third progressive phase refractory to steroids, mercaptopurine, and leucocristine. So far a complete remission has existed for 16 months after grafting.

Establishment of Specific Immune Tolerance Associated with Chimerism so as to make Tissue and Organ Transplantations Possible

A subject (A) who is the recipient of a graft of haemopoietic cells from (B) tolerates any tissue from (B), regardless of whether the haemopoietic cells were grafted at birth (Billingham *et al.*, 1953) or after total body x -irradiation in adult life. This has been demonstrated for the skin (Main and Prehn, 1955)

and the ovary (Amand and Smith, 1959) in mice, and the kidney in dogs (Mannick *et al.*, 1959).

In man the first demonstration of such tolerance was given by the patient who was the subject of our latest experiment (Mathé *et al.*, 1963b, 1964a). To-day this patient has for five months been tolerating a skin graft from the marrow-donor whose marrow graft has been proved. It is easy to see the importance of this phenomenon for organ transplantation, and in particular kidney transplantation, because the results of attempts of this kind after sublethal total irradiation or after conditioning by chemical antimetabolites have been disappointing (Murray *et al.*, 1962; Hamburger *et al.*, 1962; Küss *et al.*, 1962a, 1962b; Shackman *et al.*, 1963; Calne, 1963).

Practical Problems and Difficulties

The above three aspects of the application of haemopoietic cell grafts are limited only by the difficulties of their practical execution.

The problems fall into two categories: (1) those concerning acceptance of the graft as such, and its failures; (2) those concerning the complication of the secondary syndrome.

Taking of a Haemopoietic Cell Graft

While some haemopoietic cell grafts demonstrably took, it is useful to consider the considerable number of failures and the reasons for them.

(1) Very Early Onset of the Secondary Syndrome

This in some cases may kill the subject before myeloid restoration is accomplished; in mice, such syndromes can be produced by adding lymphoid cells to the marrow cells or, as we have demonstrated (Amiel *et al.*, 1962b), simply by increasing the number of marrow cells injected, thereby increasing the number of immunologically competent cells. The very early syndrome is frequently observed in monkeys (De Vries *et al.*, 1961). We have encountered it in two patients who at the time of death showed signs of distinct myeloid restoration in the bone-marrow, whereas the blood was not repopulated. Post-mortem histological examination revealed all the characteristics of the secondary syndrome. This is probably due to the fact that the mixture of blood and bone-marrow obtained from the donors and transfused into the patient has a very high ratio of lymphoid to myeloid cells. It should perhaps be pointed out that the mixture contains an important proportion of blood.

(2) The Relatively Small Number of Myeloid Cells Transfused when Only a Single Donor is Used

The quantities which we have been able to obtain and transfuse in these cases never exceeded 1.5×10^{10} nucleated cells; calculation by extrapolation from the data obtained in mice shows that the optimal number of nucleated cells in man is 3×10^{10} .

In view of these findings we carried out experiments in mice in order to study the possibility of using multiple donors for the same recipient (Mathé *et al.*, 1962a, 1964a). The results indicate that, for the same total number of nucleated cells, the frequency of myeloid restoration was almost as good as with a donor very close in histocompatibility and considerably better than with a more remote donor.

We have also attempted to make simultaneous use of several donors in a human case, and this resulted in excellent restoration of the graft (Mathé *et al.*, 1963b, 1964a).

(3) Failure to Choose the Correct Donor

The mouse experiments previously mentioned demonstrated that, while the cells from all the donors seem to be involved in the early proliferation responsible for myeloid restoration, only the cells from the most compatible donor persist. This was shown by studying the specific tolerance associated with the chimerism using skin grafts from various donors; only the skin graft(s) from the nearest donor(s) persisted (Mathé *et al.*, 1962a, 1964c).

A similar result was obtained in man (Mathé *et al.*, 1963b, 1964a). The subject who had received a transfusion of a mixture of bone-marrow from six donors has been completely repopulated by the cells of one of them. They correspond to the donor, who, according to two tests to be discussed later, was considered to be nearest to the recipient. It is not difficult to envisage the possible applications of such a method in the transplantation of organs, particularly kidneys.

Even using multiple donors from whom the recipient spontaneously selects the best (one or more), it is useful to have tests available that make it possible to choose, from a long list, those who have the best chances of being accepted. The test of Brent and Medawar (1963) cannot be used in the case of leukaemic recipients and normal donors because it entails injection of the recipient's leucocytes into the donors. An additional point is that this injection, if it immunizes the donors against the recipient, may aggravate the reaction of the graft against the host (secondary syndrome).

A distinct similarity of erythrocytic phenotypes, while a necessary requirement, is not sufficient. So far, serum phenotypes do not seem to have given useful information.

The study of leucocytic antigens (Colombani *et al.*, 1963) with the aid of serum from subjects immunized by blood transfusions, seems to be of some importance; in the case of our latest trial the donor who was shown (by acceptance of his skin graft) to be acceptable had been previously identified as the nearest donor on the basis of the leucocytic antigen findings (Mathé *et al.*, 1964b).

In the case in question the donor had also been indicated as the nearest by the histocompatibility test which we have proposed (Matsukura *et al.*, 1963). The principle of this test is as follows. Let there be a recipient (R) and several possible donors (D_1), (D_2), etc. Another subject (Z) is immunized by a skin graft from (R), and a study is then made of his reactions to skin grafts from (D_1), (D_2), etc. The donor whose skin was the first to be rejected in the manner of a second-set response is considered the one nearest to (R). Verified in rabbits, this test yielded the expected results in man.

(4) Cross-immunization in Man due to Previous Blood Transfusions

This cause for failure was suggested by the contrast between the frequency of failures in leukaemics given transfusions prior to x-irradiation (Mathé *et al.*, 1959a, 1960b), and the constant success in all subjects who have never received blood transfusions—one leukaemic (Mathé *et al.*, 1963b, 1964a) and the five subjects accidentally irradiated at Vinca (Mathé *et al.*, 1959b). To verify this hypothesis, we compared in mice the frequency of myeloid restoration after x-irradiation and transfusion of allogeneic bone-marrow in animals which did and those which did not previously receive blood transfusions from the same strain as the marrow donors (Da Costa *et al.*, 1964). In animals previously given fresh whole or preserved blood, white cells, or washed red cells separated from the white cells, the marrow grafts were nearly always rejected. It seems reasonable, therefore, to avoid any blood transfusion which is not strictly necessary in subjects that are to be submitted to bone-marrow grafting.

The above are the practical problems and difficulties now encountered in terms of graft acceptance. Those which concern the complicating secondary syndrome are not less important.

The Secondary Syndrome

We have described this syndrome under various circumstances in man, and demonstrated its similarity to that observed in various animal species (mice, rabbits, dogs, and especially monkeys) (Mathé *et al.*, 1959a, 1960b, 1964a). The syndrome comprises (a) clinical features—anorexia, nausea, vomiting, diarrhoea, emaciation, hepatomegaly, erythrodermia of a desquamative type, and various infections; (b) haematological changes—lymphocytopenia and eosinophilia; (c) histological changes—aplasia of the intestinal crypts, necroses, hyperkeratotic, parakeratotic, and acanthotic skin lesions, infiltration of cells into the dermis, and lymphoid (lymphocytic) aplasia with proliferation of basophilic histiocytes, the cytoplasm of which is rich in ribosomes and has no endoplasmic reticulum (Binet and Mathé, 1962; Küss *et al.*, 1962a, 1962b); and (d) biochemical anomalies—changes in serum γ -globulin and various serum enzyme levels, particularly of hepatic origin.

Because it is particularly severe in man, this syndrome constitutes the principal obstacle to the routine use of allogeneic haemopoietic cell grafts. Many efforts have been made to overcome this stumbling-block. We have confirmed, in mice, the efficacy of treatment with antimetabolites such as amethopterin and cyclophosphamide (Mathé, Amiel, and Niemetz, 1962), and the importance in man of symptomatic treatment (maintenance of aseptic conditions, antibiotics, ε -amino-caproic acid, adrenocortical steroids). In preventive treatment, we have failed in mice to reduce the frequency and severity of the syndrome by small-dose irradiation of cells before injections. The doses which reduce the secondary syndrome-inducing power of a haemopoietic graft also reduce its powers of myeloid restoration (Amiel *et al.*, 1962a).

On the other hand, we observed that preservation of haemopoietic cells in Tyrode solution for two hours at 37° C. considerably reduced the frequency of secondary syndromes without appreciably reducing that of myeloid restoration (Mathé *et al.*, 1963). An analysis of the phenomenon on the cellular level has shown that immunologically competent cells are very sensitive to this mode of preservation, which does not affect the myeloid tissue cells (Amiel and Mathé, 1963). An attempt to use this in a human bone-marrow application has meanwhile failed (Mathé *et al.*, 1964b).

The use of multiple donors has also proved an aid in preventing a secondary syndrome (Mathé *et al.*, 1962a, 1964c). In the above-mentioned experiment in mice, after total body x-irradiation at a lethal dosage and bone-marrow from five donors (some near to, some far from, the recipient in histocompatibility), the frequency of secondary syndromes for a given number of cells administered was much lower than that in controls transfused only from the most remote donor, and hardly higher than that observed in controls transfused only from the nearest donor. The explanation of this phenomenon lies in the spontaneous selection of the best donor, which operates secondarily and has been demonstrated above by tests of tolerance to the skin graft. The use of multiple donors in our latest human experiment was followed by a secondary syndrome which, though certainly severe, could be controlled simply by symptomatic treatment (Mathé *et al.*, 1963b, 1964a).

The future prospects of using haemopoietic cell grafts in man are, in our opinion, encouraging because by virtue of parallel experimental laboratory studies and clinical trials the various stumbling-blocks are being removed one by one and progress is undoubtedly being made from year to year.

REFERENCES

- Amand, W. St., and Smith, L. H. (1959). *Nature (Lond.)*, **184**, 1503.
 Amiel, J. L., and Mathé, G. (1960). *Experientia (Basel)*, **16**, 563.
 — (1963). *Nature (Lond.)*, **200**, 1224.
 —, Seman, G., Matsukura, M., and Mathé, G. (1962a). *Rev. franç. Étud. clin. biol.*, **7**, 974.
 —, Tenenbaum, R., Mery, A. M., Matsukura, M., and Mathé, G. (1962b). *Ibid.*, **7**, 970.
 Barnes, D. W. H., and Loutit, J. F. (1957). *Brit. J. Haemat.*, **3**, 241.
 Beilby, J. O. W., Cade, I. S., Jelliffe, A. M., Parkin, D. M., and Stewart, J. W. (1960). *Brit. med. J.*, **1**, 96.
 Billingham, R. E., Brent, L., and Medawar, P. B. (1953). *Nature (Lond.)*, **172**, 603.
 Binet, J. L., and Mathé, G. (1962). *Ann. N.Y. Acad. Sci.*, **99**, 426.
 Brent, L., and Medawar, P. B. (1963). *Brit. med. J.*, **2**, 269.
 Bridges, J. B., Bridges, J. M., Edelstyn, G. J. A., Lyons, A. R., and Nelson, M. G. (1960). *Lancet*, **1**, 629.
 Calne, R. Y. (1963). *Renal Transplantation*, vol. 1. Arnold, London.
 Colombani, J., Dausset, J., and Preaux, J. (1963). *Nouv. Rev. franç. Hémat.*, **3**, 499.
 Da Costa, H., Amiel, J. L., and Mathé, G. (1964). *Vox Sang. (Basel)*, **9**, 420.
 De Vries, M. J., Crouch, B. G., Van Putten, L. M., and Van Bekkum, D. W. (1961). *J. nat. Cancer Inst.*, **27**, 67.
 — and Vos, O. (1958). *Ibid.*, **21**, 117.
 Hamburger, J., Vaysse, J., Crosnier, J., Auvert, J., Lalanne, Cl. M., and Dormont, J. (1962). *Rev. franç. Étud. clin. biol.*, **7**, 20.
 Kurnick, N. B., Feder, B. H., Montano, A., Gerdes, J. C., and Nakamura, R. (1959). *Ann. intern. Med.*, **51**, 1204.
 Küss, R., Legrain, M., Mathé, G., Nedey, R., and Camey, M. (1962a). *Postgrad. med. J.*, **38**, 528.
 — (1962b). *Rev. franç. Étud. clin. biol.*, **7**, 1048.
 Lorenz, E., Uphoff, D., Reid, T. R., and Shelton, E. (1951). *J. nat. Cancer Inst.*, **12**, 197.
 McFarland, W., Granville, N. B., and Dameshek, W. (1959). *Blood*, **14**, 503.
 Main, J. M., and Prehn, R. T. (1955). *J. nat. Cancer Inst.*, **15**, 1023.
 Mannick, J. A., Lochte, H. L., Ashley, C. A., Thomas, E. D., and Ferrebee, J. W. (1959). *Surgery*, **46**, 821.
 Mathé, G. (1959). *Biological Problems of Grafting*, **1**, 314. Univ. Liège, Liège.
 — (1962). *Cancer, Leucémie et Radiobiologie*, **1**, 86. Gauthier-Villars, Paris.
 — and Bernard, J. (1958). *Bull. Ass. franç. Cancer*, **45**, 289.
 — (1959). *Rev. franç. Étud. clin. biol.*, **4**, 442.
 —, Schwarzenberg, L., Larrieu, M. J., Lalanne, Cl. M., Dutreix, A., Denoix, P. F., Surmont, J., Schwarzmänn, V., and Céoara, B. (1959a). *Rev. franç. Étud. clin. biol.*, **4**, 675.
 —, Jammet, H., Pentic, B., Schwarzenberg, L., Duplan, J. F., Maupin, B., Lataret, R., Larrieu, M. J., Kalic, D., and Djukic, Z. (1959b). *Ibid.*, **4**, 226.
 —, Amiel, J. L., and Bernard, J. (1960a). *Bull. Ass. franç. Cancer*, **47**, 331.
 —, Bernard, J., de Vries, M. J., Schwarzenberg, L., Larrieu, M. J., Lalanne, Cl. M., Dutreix, A., Amiel, J. L., and Surmont, J. (1960b). *Rev. Hémat.*, **15**, 115.
 —, Amiel, J. L., Matsukura, M., and Mery, A. M. (1962a). *C.R. Acad. Sci.*, **255**, 3480.
 — and Niemetz, J. (1962b). *Ibid.*, **254**, 3603.
 — and Friend, Ch. (1962c). *Bull. Ass. franç. Cancer*, **49**, 416.
 — and Schwarzenberg, L. (1963a). *Ann. N.Z. Acad. Sci.* In press.
 — and Mery, A. M. (1963b). *Blood*, **22**, 44.
 —, Cattani, A., and Schneider, M. (1963c). *C.R. Acad. Sci.*, **257**, 3527.
 —, Cattani, A., Schneider, M., de Vries, M. J., Tubiana, M., Lalanne, Cl., Binet, J. L., Papiernik, M., Seman, G., Matsukura, M., Mery, A. M., Schwarzmänn, V., and Flaisler, A. (1964a). *Blood*. In press.
 —, de Vries, M. J., Amiel, J. L., Schwarzenberg, L., Cattani, A., Schneider, M., Nordman, R., and Schwarzmänn, V. (1961). *Rev. Europ. Cancer*. In press.
 —, Matsukura, M., Mery, A. M. (1964c). *Brit. J. Haemat.*, **10**, 257.
 Matsukura, M., Mery, A. M., Amiel, J. L., and Mathé, G. (1963). *Transplantation*, **1**, 61.
 Murray, J. E., Merrill, J. P., Dammin, G. J., Dealy, J. B., Alexandre, G. W., and Harrison, J. H. (1962). *Ann. Surg.*, **156**, 337.
 Papiernik-Berkhauer, M., Amiel, J. L., and Mathé, G. (1963). *C.R. Acad. Sci.*, **256**, 5232.
 Russell, E. S., Bernstein, S. E., Lawson, F. A., and Smith, L. J. (1959). *J. nat. Cancer Inst.*, **23**, 557.
 Shackman, R., Dempster, W. J., and Wrong, O. M. (1963). *Brit. J. Urol.*, **35**, 222.
 Thomas, E. D., Lochte, H. L., Cannon, J. H., Sahler, O. D., and Ferrebee, J. W. (1959). *J. clin. Invest.*, **38**, 1709.
 Tran Ba Loc, Mathé, G., and Bernard, J. (1958). *Rev. franç. Étud. clin. biol.*, **3**, 461.
 Uphoff, D. (1963). *J. nat. Cancer Inst.* In press.
 Van Bekkum, D. W., Vos, O., and Weyzen, W. W. H. (1956). *Rev. Hémat.*, **11**, 477.
 Woodruff, M. F. A., and Symes, M. O. (1962). *Brit. J. Cancer*, **16**, 707.