PEGG * BONE MARROW TRANSPLANTATIO LUKE

Bone Marrow Transplantation

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FOREWORD

The study of bone marrow transplantation which has occupied many workers in recent years has now produced a very considerable literature. The author of this monograph has been actively concerned in this field himself to which he has contributed much original work and even more original equipment. In this monograph he has collected a most extensive bibliography of the experiments concerning the work performed on laboratory animals and in the human, and the text has been so arranged that it can be readily understood by those with but a slender knowledge of the subject. The clinical applications of bone marrow transplantation have been thoroughly explored by the author and his associates and his conclusions as to its efficacy are clearly drawn. A feature of the work are the tables and illustrations which are a model of clarity. It is not surprising to find that his knowledge of the behaviour of bone marrow cells at low temperature has led him to take a leading part in the foundation of the Society for Low Temperature Biology in this country. The foundation of such a society shows the present great interest in low temperature biology and its application to all kinds of living systems. It has given us great pleasure to have been associated with him in this work and we would like to emphasise the diligence, technical skill and inventive acumen which he has displayed.

J. G. HUMBLE K. A. NEWTON

PREFACE

In recent years, the transplantation of haemopoietic tissue has assumed considerable importance, particularly since there is a real possibility of useful clinical applications. Knowledge of fundamental processes involved in bone marrow grafting is now sufficiently extensive to warrant the preparation of a small monograph to collect together the basic scientific information and relate it to those clinical therapeutic attempts which have been made so far. This monograph has, therefore, been written primarily for haematologists and for physicians with a particular interest in haematology, but although the main emphasis is on the clinical application of haemopoietic tissue grafting, the fundamental experimental work is also dealt with at some length and is discussed in relation to the clinical work. Similarly, the problems of low temperature storage are dealt with quite fully, since bone marrow storage forms an indispensable part of any clinical haemapoietic transplantation programme. An attempt has been made to bring together as much as possible of the relevant literature, and it is intended that the bibliographies should be of value to those wishing to refer extensively to the original literature.

It may be useful to define at this point some of the transplantation terminology used in the monograph. A *syngeneic* transplant is one where both donor and recipient belong to the same inbred strain or are identical twins; there is no immune response by the recipient against the graft and consequently no homograft reaction. *Allogeneic* transplants are made between animals of the same species where genetic differences between host and donor do exist and in fact normally result in a homograft reaction and graft rejection. When host and donor belong to different *species* the term *xenogeneic* is used. When a haemopoietic graft has been successfully made, and cells of donor type are to be found in the circulating blood of the graft recipient, the animal is known as a *chimera*.

Of the many colleagues who have collaborated in the studies which form the basis of the monograph, I am particularly indebted to Doctors J. G. Humble and K. A. Newton, whose help and guidance have been invaluable. The entire investigation has been

supported by the British Empire Cancer Campaign for Research, to whom I express my gratitude.

I am very grateful to the following investigators who allowed me to make use of illustrations previously published by themselves. Dr. H. Chaplin, Jr. (Fig. 39), Mr. Peter Clifford (Fig. 16), Dr. A. Dunjic (Fig. 4), Dr. P. F. Harris (Fig. 2), Dr. H. B. Hewitt (Fig. 3), Dr. J. G. Humble (Figs. 12, 13 and 19), Dr. A. L. Kretchmar (Fig. 15), Professor G. Mathé (Figs. 5 and 8), and Dr. M. J. Ashwood-Smith (Fig. 24); these illustrations were first published in *Acta Haematologica* (Fig. 2), *Blood* (Figs. 5 and 39), *The Lancet* (Fig. 16), *Nature* (Figs. 3, 4, and 24), *New England Journal of Medicine* (Fig. 15), *Proceedings of the International Symposium on Bone Marrow Therapy and Chemical Protection in Irradiated Primates* (Figs. 12, 13 and 19), and *Revue Française d'Études Cliniques et Biologiques* (Fig. 8), to all of whom I wish to express my thanks for their kind co-operation. I am particularly indebted to Drs. J. A. Armstrong and J. Farrant of the National Institute for Medical Research, London, for their permission to reproduce an electron micrograph not previously published (Fig. 23), to Dr. E. H. Porter for Fig. 17 and to Matburn Research Ltd. and Union Carbide Ltd. for photographs of cooling equipment (Figs. 44 and 46).

In the preparation of this monograph considerable use has been made of material previously included in an M.D. thesis of the University of London. I also acknowledge the permission of the Editors and Publishers of the following journals to reproduce material which I have previously published in them: *British Journal of Cancer* (Figs. 14, 18 and Tables 1–7 inclusive), *Cancer Chemotherapy Reports* (Fig. 22), *Cryobiology* (Figs. 17 and 41), *Journal of Applied Physiology* (Figs. 25–32 inclusive, and 34–36 inclusive), *Journal of Clinical Pathology* (Figs. 42 and 43), *The Lancet* (Figs. 37 and 38), *Postgraduate Medical Journal* (Figs. 10 and 11).

The illustrations were prepared by the Department of Medical Illustration, Westminster Medical School. I am especially grateful to Mrs. M. Chatfield whose enthusiastic secretarial assistance has contributed immeasurably to the preparation of the manuscript.

D. E. PEGG

January, 1966

CONTENTS

CHAPTER I

THE BONE MARROW— PRELIMINARY CONSIDERATIONS

Structure

After birth, blood formation is normally restricted to the bone marrow, and by the time adult life is reached haemopoietic marrow is found only in the bones of the skull, thoracic cage, pelvis and vertebral column. The medullary cavity of these bones is lined by endosteum and contains a connective tissue matrix supporting the marrow vascular system. The nutrient artery to each bone divides to form arterioles and capilliaries which supply a complex system of sinusoids; these ultimately drain by way of venules into the vein accompanying the nutrient artery. The extravascular space within the marrow cavity contains a variable number of fat cells, but is predominantly occupied by the haemopoietic tissue, which can, for descriptive purposes, be divided into erythropoietic, leucopoietic and thrombopoietic compartments, although these are extensively inter-mixed in the marrow. The earliest identifiable cell of the erythroid series is the **proerythroblast**, a large basophilic cell with a finely stippled nucleolated nucleus, which differentiates through *early* and *intermediate* forms into the **late normoblast**; this differentiation is accomplished by a reduction in size, loss of nucleoli, condensation of the nuclear chromatin, loss of ribonucleic acid and acquisition of haemoglobin in the cytoplasm. Several cell divisions probably occur during this process of differentiation. The late normoblast becomes a **reticulocyte** by loss, probably extrusion of its pyknotic nucleus, and is released into the blood in this form, and then after 1–2 days the basophilic reticulum is lost and the mature **erythrocyte** is produced. The most primitive cell of the myeloid series is the **myeloblast**, a large cell with scanty deeply basophilic cytoplasm, a finely reticulated nucleus and several nucleoli. This cell differentiates, again with several mitoses, to form the **promyelocytes**, **myelocytes**, **meta-myelocytes** and mature **granulocytes**; this is accomplished by reduction in size, loss of nucleoli and acquisition of faintly acidophilic cytoplasm containing characteristic granules—either neutrophilic, acidophilic or baso-

philic. The mature cells have lobulated nuclei. Platelets are formed by direct budding into the vascular channels of cytoplasmic fragments from the **megakaryocytes**; these very large granular cells with extensively lobulated nuclei develop from smaller agranular cells with lobed nuclei. All the cells mentioned so far account for between 75 and 95 per cent of the total marrow cells, the ratio of myeloid to erythroid cells commonly being between 4:1 and 10:1. The remaining cells are described as lymphocytes (5–20 per cent), monocytes (0–5 per cent) and primitive undifferentiated cells (0–1 per cent) and it must be among these that the "stem" cells, from which the recognisably myeloid and erythroid cells are derived, are to be found. The precise identity of the stem cell is, however, still a matter of debate, and it is even uncertain whether or not the myeloid and erythroid series are derived from a common source; transplantation studies have, in fact, provided conflicting evidence on this point. There is, however, reasonable evidence to support the suggestion that the resting stem cell is morphologically indistinguishable from the small lymphocyte, although this is not the same thing as saying that all small lymphocytes are potential stem cells. According to Lajtha (1960*a*) about 3–4 per cent of the bone marrow cells are stem cells, and since the primitive undifferentiated cells never reach such a high proportion under normal circumstances, it is impossible to avoid the conclusion that the lymphocyte-monocyte group must include the stem cells: the primitive undifferentiated cells may comprise, or at any rate include, that part of the stem cell compartment which is actively dividing.

Function

The only known function of the bone marrow is the production of blood cells by the processes of division and maturation outlined above. The precise mechanism by which this occurs, has, however, been the subject of considerable controversy (Fig. 1). It has been argued, notably by Osgood (1959) and by Loutit (1960) that the stem cell compartment is maintained by symmetrical division, i.e. one stem cell dividing to produce two similar stem cells, but that the myeloid and erythroid compartments are fed by occasional asymmetrical divisions in which a stem cell divides to produce one stem cell and one cell destined to differentiate. As Lajtha has pointed out (Lajtha, 1960*b*) this is a most improbable concept, since it implies that during division one cell receives something (which causes it to differentiate) which the other cell does not.

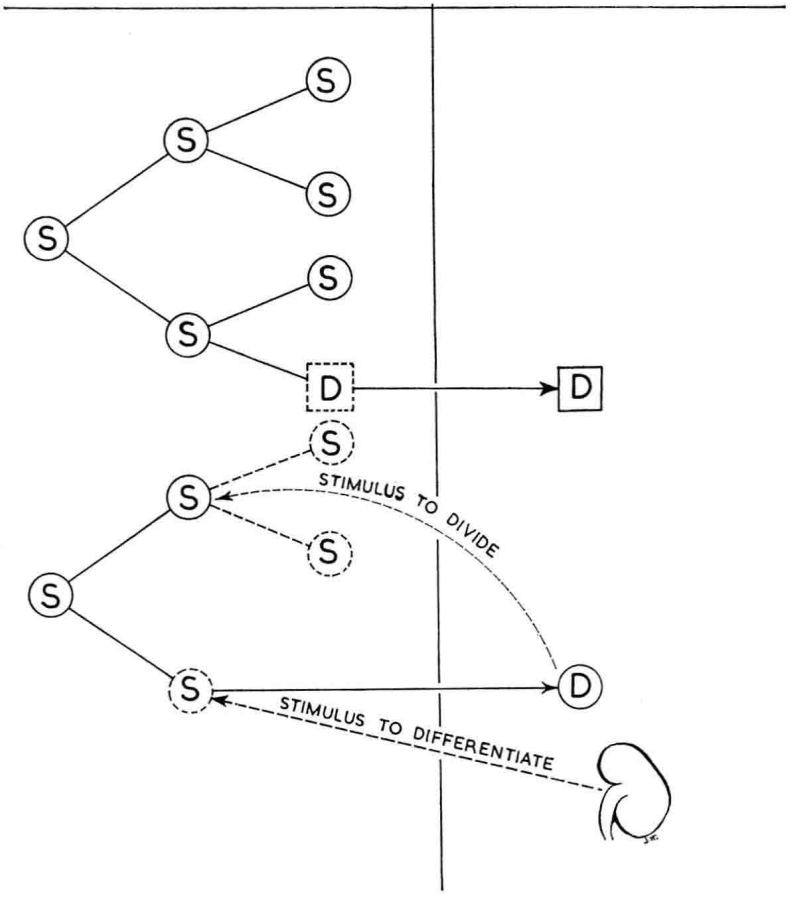

FIG. 1.—Schemes of bone marrow kinetics.

Above: Osgood's scheme. Cell [D] differentiates because it is intrinsically different from sister cell (S); differentiation is 'built-in'.

Below: Lajtha's scheme. Cell (S) differentiates because it receives the specific biochemical stimulus to do so. The sister cell (S) is identical, but it *divides* because it receives a specific biochemical stimulus to do so.

Lajtha and his associates (Lajtha and Oliver, 1960; Lajtha *et al.*, 1962, 1964) have developed several more sophisticated models of marrow function which avoid this proposition and account for a remarkable range of observations of bone marrow kinetics in

various circumstances. In principle, these workers propose that the bone marrow stem cell compartment has its own feed-back mechanism to control its size so that when any number of cells is removed from the compartment, each departing cell triggers off one remaining cell to divide—this cell divides symmetrically and restores the compartment to its original size. The signal for the cells to leave the compartment is humoral; in the case of the erythroid series it is erythropoietin. Lajtha and Oliver (1960) have shown how this type of model can account for the normal regulation of erythropoiesis, recovery from radiation damage and various other phenomena observed in bone marrow disorders.

The Total Amount of the Bone Marrow

Since bone marrow is scattered so widely throughout the body it is no easy matter to determine its total amount. However, it is most interesting from a theoretical view-point to do so, and when we come to consider some of the more important quantitative aspects of marrow transplantation it will be essential to have these data to hand. Consequently, we must look in some detail at the methods which have been used to determine the total marrow cellularity of man and the relevant experimental animals, and assess the reliability of the estimates obtained. Three methods, all indirect, have been used to obtain these data; they may be designated the *anatomical method*, the *erythrokinetic method* and the *radioiron method.*

The Anatomical Method

In this technique, the unit cellularity of bone marrow is multiplied by the estimated total marrow volume. The unit cellularity of rat marrow has been estimated by a histological technique involving the enumeration of cells in sections of known thickness (Kindred, 1942) and by the more accurate method of counting cells in suspensions made from known weights or volumes of marrow (Fruhman and Gordon, 1953; Meineke and Crafts, 1956; Awaya *et al.*, 1960). The two estimates for Wistar rats agree well (1·75 and 1·81 $\times$ 10^6/cu.mm.). Similar techniques have been used to measure the cellularity of marrow removed from the dog, and the guinea-pig. In the case of the mouse it was found that the amount of marrow which could be obtained was so small that it was necessary to measure the volume of the cavity from which the cells had been obtained in order to derive the unit cellularity (Pegg,

1962). Accordingly, the femora of freshly killed CBA mice were gently excised and the epiphyses cleanly removed. The marrow cells were washed out of each shaft and counted, and the volumes of the shaft cavities were determined by direct measurement under a low power microscope. From these data the unit cellularity was calculated at $2{\cdot}41 \times 10^6$ cells/cu.mm. (SD $0{\cdot}20 \times 10^6$ cells/cu.mm.). (See Table I.)

Total marrow volume was first measured accurately by Mechanik (1926) who macerated human bones and noted their change in dry weight; a value of 4·6 per cent of the total body weight was obtained. The agar impregnation technique of Nye (1931) was a considerable technical improvement: he filled the marrow-containing bones of the rabbit with agar and noted their increase in solid volume. A value of 1·70 per cent of the body weight was obtained. The same technique was used by Fairman and Whipple (1933) and their figures lead to an estimate of 1·65 per cent of body weight for the total haemopoietic marrow of adult mongrel dogs. A very painstaking study using an improved agar technique led Hudson (1958) to estimate the total haemopoietic marrow volume of the guinea-pig at 1·56 per cent of the body weight. The first estimate of rat marrow volume was 3·0 per cent of the body weight (Fairman and Corner, 1934), but more recent estimates have both been 1·65 per cent (Hashimoto, 1953; Watanabe, 1955). It is interesting to note that the marrow volume of all these animals falls in the narrow range of 1·56–1·70 per cent of the body weight, with a mean of 1·65 per cent, and one therefore seems justified in using this value to calculate the total marrow cellularity of the mouse—the only animal where a direct determination of total marrow volume has not been made (owing to the small size of the bones, surface tension effects render the agar impregnation technique impracticable).

The anatomical method can also be applied to man: the results obtained in the collection of bone marrow from excised ribs (see Chapter IX) provide the basis for the calculation, and the additional data required (the marrow volume of a rib, the total haemopoietic marrow volume, and the relative cellularity of rib and other bone marrow) are all available. The volume of the ribs used is in fact 9·3 ml. (Mechanik, 1926; Ellis, 1961), giving a cellularity of $1{\cdot}29 \times 10^5$ cells/cu.mm. Custer (1932) has shown that rib marrow in adults is only about 30 per cent as cellular as marrow contained in other sites, and the cellularity elsewhere may therefore be

estimated at $4{\cdot}3 \times 10^5$ cells/cu.mm. Mechanik's figure for the total marrow cavity is 4·6 per cent of the body weight, and using the analysis of Mechanik's data provided by Woodard and Holodny (1960) the total *haemopoietic marrow* volume is 1,500 ml. for a 70 kg. man, of which 240 ml. is contributed by the ribs. If these volumes are multiplied by the unit cellularities given above, we obtain a total of $8{\cdot}1 \times 10^9$ cells/kg.

The anatomical method permits the calculation of the total marrow cellularity of the mouse, rat, guinea-pig, dog and man.

The Erythrokinetic Method

Kindred (1942) suggested that consideration of red cell production could be used to calculate total marrow cellularity, but as Patt (1957) pointed out, the data available for these calculations were not very precise. Osgood (1954) calculated the total human bone marrow cellularity from an assumed red cell production rate, a normoblast maturation time, the myeloid : erythroid ratio and the ratio of proliferating to differentiating normoblasts. His result (46×10^9 cells/kg.) was an overestimate because the marrow reticulocyte stage was ignored; if this is allowed for on the basis of two marrow reticulocytes for each differentiating normoblast (Patt, 1957) the estimate becomes $9{\cdot}3 \times 10^9$ cells/kg. This is in quite good agreement with Patt's own calculation of $13{\cdot}4 \times 10^9$ cells/kg. which was based on red cell output, mitotic index, the duration of mitosis and assumed proportions of marrow reticulocytes, proliferating normoblasts and proliferating myeloid cells. The principal uncertainty of this method lies in the assumed duration of mitosis, although when the same procedure is used to calculate the marrow cellularity of the rat and the dog some of the other data are also of doubtful accuracy.

The erythrokinetic method has enabled the total marrow cellularity of man, the rat and the dog to be calculated. Patt's procedure gives the total of the erythroid and the myeloid cells: in Table I 10 per cent has been added to allow for other cells present.

The Radioiron Method

Suit (1957) showed that the radioactive isotope of iron, ^{59}Fe could be used to measure total marrow cellularity in man. He assumed that when an ^{59}Fe salt is injected intravenously the amount of ^{59}Fe present in the total bone marrow 24 hours later is the same as that present in the total red cell mass on the eighth day;

consequently the ^{59}Fe taken up by the marrow can be measured. If a marrow sample is taken at 24 hours, the ratio of its cell content and ^{59}Fe activity will be the same as the ratio of the total marrow cellularity and the total ^{59}Fe in the whole body marrow at 24 hours. An allowance was made for uptake of ^{59}Fe by reticulocytes, and the total marrow cellularity calculated. Suit recognised that some of these assumptions were approximate, but it seems that the error in assessing the proportion of the injected ^{59}Fe taken up by the marrow is considerable, since it is known that some of the iron originally cleared to the stores is utilised for haemoglobin synthesis during the 8-day period (Finch *et al.*, 1949). Donohue *et al.* (1958 *a* and *b*) used a fixed value of 66 per cent for the proportion of iron taken up by the bone marrow at the time of minimum blood activity (18 hours); this figure was obtained by direct measurement in animal carcases and seems to be preferable to Suit's assumption.

Suit measured the total erythroid cellularity of six patients with malignant disease whom he thought "should not be considered normal". The mean value was 16×10^{10} red cell precursors with a considerable range; assuming a myeloid-erythroid ratio of 3:1 and a body weight of 70 kg. this gives a total cellularity of $9{\cdot}1 \times 10^9$ cells/kg.

Donohue used the technique to measure the marrow cellularity of man, the rhesus monkey, the rat and the rabbit, but his radioactivity measurements were made on centrifuged marrow samples, ignoring the radioactivity present in the supernatant. This activity was shown to be derived from the cytoplasm of cells which were probably included in the nucleated cell counts. Donohue's results are therefore overestimates, but since the percentage of the activity contained in the supernatant is specified it is possible to recalculate the results to allow for this.

The error introduced by reticulocyte ^{59}Fe uptake is negligible when surgically excised marrow is used. When the marrow sample has been obtained by needle puncture, its radioactivity must be corrected for the presence of reticulocytes in proportion to its red cell content. The inclusion of marrow reticulocytes with a higher iron-uptake introduces an error here (Suit, 1957), but because of the gross dilution of marrow reticulocytes by blood reticulocytes this is less than 1 per cent. When dealing with aspirated marrow it is of course also necessary to correct the total nucleated cell count for dilution with peripheral blood leucocytes: this can be

TABLE I

TOTAL BONE MARROW CELLULARITY OF VARIOUS EXPERIMENTAL ANIMALS AND MAN

Species	Animal Strain	Method	Nature of Report	Total marrow cellularity (cells per kg. body weight) Published value	Recalculated value	Accepted value	Reference
Mouse	C3H	Anatomical	Measured cellularity = $1{\cdot}80 \times 10^6$ cells/cu.mm. Assumed mass = 2·0 per cent body weight	$36{\cdot}0 \times 10^9$	—	—	50
	CBA	Anatomical	Measured cellularity = $2{\cdot}41 \times 10^6$ cells/cu.mm. Assumed mass = 1·65 per cent body weight	$39{\cdot}8 \times 10^9$	—	$39{\cdot}8 \times 10^9$	45
Rat	(Albino)	Anatomical	Measured cellularity = $2{\cdot}0 \times 10^6$ cells/cu.mm. Measured volume = 1·20 per cent body volume	$24{\cdot}0 \times 10^9$	—	—	25
	Wistar	Anatomical	Measured volume = 3·0 per cent body weight	—	—	—	14
		Anatomical	Measured mass = 1·65 per cent body weight	—			22
		Anatomical	Measured mass = 1·65 per cent body weight	—	$29{\cdot}4 \times 10^9$ for Wistar	$29{\cdot}4 \times 10^9$ for Wistar	51
	Wistar	Anatomical	Measured cellularity = $1{\cdot}75 \times 10^6$ cells/cu.mm.	—			36
	Wistar	Anatomical	Measured cellularity = $1{\cdot}81 \times 10^6$ cells/cu.mm. Assumed mass = 1·65 per cent body weight	$29{\cdot}9 \times 10^9$			1
	Sprague-Dawley	Anatomical	Measured cellularity = $2{\cdot}00 \times 10^6$ cells/cu.mm.	—	—	—	36
	Long Evans	Anatomical	Measured cellularity = $1{\cdot}78 \times 10^6$ cells/cu.mm.	—	—	—	36
	Long Evans	Anatomical	Measured cellularity = $2{\cdot}40 \times 10^6$ cells/cu.mm.	—	—	—	19
	—	Erythro-kinetic	Myeloid and erythroid cellularity = $49{\cdot}0 \times 10^9$ cells/kg.	$49{\cdot}0 \times 10^9$	$54{\cdot}0 \times 10^9$	—	44
	Sprague-Dawley	Radioiron	Total marrow cellularity = 13–22 $\times 10^9$ cells/kg.	$17{\cdot}0 \times 10^9$	$12{\cdot}0 \times 10^9$	—	11

Guinea-pig	—	Anatomical	Measured cellularity = $1{\cdot}60 \times 10^6$ cells/cu.mm.	—	—		16
	Dunklin-Hartley	Anatomical	Measured cellularity = $1{\cdot}80 \times 10^6$ cells/cu.mm.	—	—	$26{\cdot}4 \times 10^9$	54
	Dunklin-Hartley	Anatomical	Measured mass = 1·56 per cent of the body weight	—	—		23
Rhesus monkey		Radioiron	Total marrow cellularity = 22–24 $\times 10^9$ cells/kg.	$34{\cdot}0 \times 10^9$	$24{\cdot}0 \times 10^9$	$24{\cdot}0 \times 10^9$	11
Dog	(Mongrel)	Anatomical	Measured cellularity = $1{\cdot}37 \times 10^6$ cells/cu.mm.	—			47
	(Mongrel)	Anatomical	Measured marrow mass = 2·2 per cent body weight (red and yellow marrow)	—	$22{\cdot}7 \times 10^9$	$21{\cdot}4 \times 10^9$	15
	(Mongrel)	Anatomical	Measured erythropoietic marrow = 75 per cent total marrow	—			40
	(Mongrel)	Erythro-kinetic	Myeloid and erythroid cellularity = $18{\cdot}2 \times 10^9$ cells/kg.	$18{\cdot}2 \times 10^9$	$20{\cdot}0 \times 10^9$		44
Man		Anatomical	Total marrow cellularity = $8{\cdot}1 \times 10^9$ cells/kg.	$8{\cdot}1 \times 10^9$			45
		Erythro-kinetic	Myeloid and erythroid cellularity = $46{\cdot}0 \times 10^9$ cells/kg.	$46{\cdot}0 \times 10^9$	$10{\cdot}2 \times 10^9$		41
		Erythro-kinetic	Myeloid and erythroid cellularity = $12{\cdot}1 \times 10^9$ cells/kg.	$13{\cdot}4 \times 10^9$		$10{\cdot}9 \times 10^9$	44
		Radioiron	Erythroid cellularity = 16×10^{10} cells/patient	—	$9{\cdot}2 \times 10^9$		49
		Radioiron	Total marrow cellularity = $18{\cdot}0 \times 10^9$ cells/kg.	$18{\cdot}0 \times 10^9$	$13{\cdot}0 \times 10^9$		11
		Radioiron	Total marrow cellularity = $9{\cdot}3 \times 10^9$ cells/kg.	$11{\cdot}5 \times 10^9$			45
		Radioiron	Total marrow cellularity = $10{\cdot}9 \times 10^9$ cells/kg.	$10{\cdot}9 \times 10^9$			21

done by subtraction after correction for differences in red cell content. All these precautions and corrections were attended to in measuring the total marrow cellularity of eight patients with malignant disease and a mean result of $11{\cdot}5 \times 10^9$ cells/kg. (SD $5{\cdot}3 \times 10^9$) was obtained (Pegg, 1962).

Harrison (1962) has used the same technique to measure the marrow cellularity of 10 haematologically normal patients and 11 cases of disseminated malignant melanoma. In the 10 normal cases, marrow was removed both by excision of a rib and by needle aspiration: the agreement obtained between the two confirms the validity of the corrections discussed above. Harrison's mean result of $10{\cdot}9 \times 10^9$ cells/kg. based on 31 measurements in 21 patients is probably the most reliable estimate of human total marrow cellularity available at this time.

The ^{59}Fe method has been used to calculate the total marrow cellularity of the rat, the rhesus monkey, the rabbit (which we need not consider here) and man.

The various techniques used to measure total marrow cellularity have given remarkably good agreement. The least satisfactory estimate is that for the rat, but this may be, at least partly, due to real variation between strains (Meineke and Crafts, 1956). Nevertheless, the anatomical estimates for the Wistar rat used in the radiation experiments to be considered later are in good agreement.

It appears that marrow cellularity decreases relative to body weight with increasing weight not only within individual species (Fand and Gordon, 1957), but also between species—see Table I. Even so, human marrow cellularity expressed in terms of body weight is surprisingly low: this could be due to longer peripheral survival of the formed blood cells, but reliable data to test this hypothesis do not appear to be available.

Early Experiments in the Therapeutic Use of Bone Marrow

The earliest attempts to use bone marrow therapeutically appear to have been made by Brown-Séquard and d'Arsonaval in 1891 (Quine, 1896): they administered marrow, apparently by mouth, in cases characterised by defective blood formation, notably in leukaemia. This report was soon followed by others making claims for the effectiveness of orally administered red-marrow extracts in pernicious anaemia (Kast and Rumpel, 1892; Fraser, 1894; Danforth, 1894). These physicians all used a glycerol extract of