CHAPTER I

History of the Radiation Chimaera

The term "radiation chimaera" was introduced by Ford *et al.*[143] (1956), to designate an animal which carries a foreign haemopoietic system, as a result of whole body irradiation followed by transplantation of haemopoietic cells derived from another animal.

It is impossible to state exactly when the first radiation chimaeras appeared on the scene in experimental biology, because this amazing product of radiation research was not recognised as such until about 1955.

The line of investigation which led to its discovery was initiated some time before 1949. In that year Jacobson *et al.*[193] published the results of a study on the protection of mice given an otherwise lethal dose by shielding of the spleen during the irradiation. This procedure caused an impressive reduction in mortality, and moreover the spleen appeared to be specific in this respect since shielding of other organs was much less effective, the only notable exception being the shielding of a whole leg.

In the mouse the spleen is essentially a haemopoietic organ and these results underlined the significance of the damage to the haemopoietic system for the development of that form of radiation sickness which is now generally known as "the bone marrow syndrome".*

In the experiments described above the spleen was exteriorised in order to permit effective and selective shielding and the investigators made every attempt to maintain the normal blood supply to the organ. In some cases, however, these attempts failed and the spleen was found to be completely infarcted after the completion of the irradiation. It was returned, nevertheless, to the abdominal cavity and subsequent observation of these animals showed them to be as equally well protected as the other animals in which the whole procedure had been technically successful.

Jacobson and his co-workers, with remarkable insight, drew the

* A description of radiation sickness and the bone marrow syndrome is given in Chapter II.

TABLE I: 1. Therapeutic effects of bone marow transplantation in irradiated animals
Data up to 1955

Recipient	Donor	Amount administered*	Survival at 21–30 days per cent	References
Mouse (CFI no. 1)	Isologous	0·1– 0·9 cells 1 – 5 (× 10^6) 5 –10 i.v. 10 –15 15 –20	12 33 55 43 40	Jacobson et *al.* (1955)[190]
Mouse (4 strains)	Isologous	1·5 mg i.v. 1·5 mg i.p.	68–100 10–75	Congdon *et al.* (1952)[98]
Mouse (C_3Hb)	Homologous (LAF_1)	1·5 mg i.v.	39	Congdon *et al.* (1952)[98]
Mouse (LAF_1)	Guinea pig	100 mg i.p. or i.v.	40–60	Lorenz *et al.* (1951)[231] Lorenz *et al.* (1952)[230] Congdon *et al.* (1952)[98]
Mouse LAF_1 and A	Rat (3 strains)	20–30 mg i.v.	0–72	Congdon and Lorenz (1954)[93]

Mouse	Dog	Not stated	None	Congdon and Lorenz (1954)[93]
Mouse	Rabbit	Not stated	None	Barnes and Loutit (1954)[28] Congdon and Lorenz (1954)[93]
Rat (Sprague-Dawley)	Isologous	50–100 mg i.v.	70	Fishler *et al.* (1954)[140]
Rat	Rat	Not stated	Increased	Chamberlain (1952)[73]
Rat	Rat	Not stated	Insignificant effect	Talbot and Gerstner (1951)[394] Lorenz and Congdon (1952)[227]
Guinea-pig (family 2, NIH)	Isologous	10 mg i.v.	85	Congdon *et al.* (1952)[98]
Guinea-pig	Guinea-pig	Not stated	Increased	Barnes and Loutit (1954)[28]
Guinea-pig	Rabbit	Not stated	None	Congdon and Lorenz (1954)[93]
Rabbit	Rabbit	2 femurs	Increased	Hilfinger *et al.* (1953)[177]
Hamster	Hamster	4 long bones and spleen of a 2 week old donor	Increased	Smith *et al.* (1955)[383]
Dog	Dog	Not stated	Equivocal effect	Rekers *et al.* (1950)[346]

* i.v., intravenous i.p., intraperitoneal

conclusion that the infarcted spleens were probably equivalent to a spleen autograft and soon afterwards they reported that the implantation of un-irradiated autologous and isologous spleen after the irradiation had a similar therapeutic effect[195].

In addition, they were able to show that the intraperitoneal injection of isologous spleen cell suspensions was therapeutically effective[189]. In the same year Lorenz *et al.*[231] showed that lethally irradiated mice and guinea pigs can be protected by the parenteral administration of isologous bone marrow after the irradiation. In subsequent papers[229, 230], these investigators reported the therapeutic efficacy of homologous and heterologous bone marrow cell suspensions and "bone brei" as measured by 20 or 30 day survival.

The application of haemopoietic cell suspensions to the treatment of irradiated animals was greatly extended in succeeding years by the groups of both Jacobson and Lorenz as well as in a number of other laboratories. Much of this work was of an exploratory nature and consisted of the testing of various recipient–donor combinations. Few investigators were concerned with the quantitative aspects of the problem, as can be seen in Tables I: 1 and I: 2 which summarise the results published up to the end of 1955.

By that time the beneficial effect of haemopoietic cells in the prevention of "bone marrow death" had been firmly established and the interests of several leading groups of investigators in this field became centred on the mechanism of this therapeutic action.

The nature of the therapeutic action of haemopoietic cells

From his early experiments Jacobson had postulated that the mouse spleen contained a *humoral factor* capable of stimulating the regeneration of blood-forming tissues. This hypothesis was undoubtedly founded on the results of the histological observations on animals with shielded spleens[192, 196]. These had revealed that an initial massive destruction of the blood-forming cells occurred to the same extent in shielded as in non-shielded mice during the first few days. Beginning on the fourth day, however, an intensive proliferation of haemopoietic cells was observed in the bone marrow of the shielded mice, followed somewhat later by a regeneration of the lymphoid tissues. Complete restoration of the bone marrow was reached on the 8th day following irradiation and in the thymus between the 12th and 15th day.

The non-shielded mice could be studied until the time of death

TABLE I: 2. Therapeutic effects of spleen cells in irradiated animals

Recipient	Donor	Amount administered*	Survival at 30 days (per cent)	Remarks	References
Mouse (LAF_1)	Isologous	"Homogenate" of 1–2 spleens injected i.p.	53–79	60–70% of the cells were disrupted	Cole *et al.* (1952)[87]
		"Homogenate" of 28–160 mg i.p.	57–100	Irradiation dose not lethal to all control groups	Cole and Ellis (1953)[81]
Mouse (CBA)	Isologous	Ground infant spleens 1/20–4 per recipient i.v.	20–100		Barnes and Loutit (1953)[27]
		4 spleens i.p.	30		Barnes and Loutit (1954)[28]
Mouse (LAF_1)	Isologous	"Homogenate" of 1 spleen i.v.	No data	Accelerated recovery (following LD_{75})	Smith *et al.* (1954)[382]
Mouse (CF_1 no. 1)	Isologous	$0{\cdot}5$–11×10^6 cells i.v.	50–72	Donors 2–5 days old	Jacobson *et al.* (1955)[190]
Mouse (LAF)	Homologous mouse LAF_2	"Homogenate" of 25–50 mg i.p.	Little or no protection		Cole and Ellis (1953)[81]
Mouse (LAF_1)	Homologous mouse A	1 spleen equivalent i.v.	56	High secondary mortality	Barnes and Loutit (1954)[28]
Rats (Sprague-Dawley)	Homologous (Sprague-Dawley)	"Homogenate" of 40–400 mg i.p. or i.v.	11–20		Cole and Ellis (1953)[81]

* i.v., intravenous i.p., intraperitoneal

around the 10th day. They showed no haemopoietic regeneration at any time or at best a few isolated groups of erythropoietic cells. Since the astonishing migratory activity of haemopoietic cells had not been recognised at that time, the humoral factor concept was the logical outcome of these observations. The majority of the investigators were inclined to explain the beneficial effects of bone marrow injections and of post-irradiation parabiosis in a similar way, because it was found that these procedures resulted in an accelerated haemopoietic recovery wholly comparable to that seen after spleen shielding or spleen implantation.

As an alternative to the humoral hypothesis it was suggested—in particular by the Harwell group led by Loutit[27, 28]—that the active principle in spleen and bone marrow suspensions might be living cells, which would act at least for some time as a tissue graft. This cellular hypothesis received little support, particularly during the years immediately following Jacobson's and Lorenz's discoveries. In 1954 Lorenz and Congdon[229] wrote in a paper describing therapeutic effects obtained by the administration of homologous and heterologous bone fragments: "Therefore, if we assume the existence of humoral factors in irradiation protection by spleen shielding and bone marrow injection as postulated first by Jacobson, osseous tissues should also contain such a factor. This factor may be the same as in bone marrow protection. The experiments in which it was shown that rat bone marrow also affords protection against irradiation injury give additional proof that bone may contain such a factor. The histological evidence shows that rat bone transplanted into irradiated mice does not form bone marrow. Yet it protects irradiated mice."

Obviously it was of extreme importance to reach a clear decision about the mechanism involved. In the case of a humoral factor its isolation, identification and perhaps even its chemical synthesis appeared to be within reach of modern biochemical technology. The availability of a large supply of this factor in a readily usable form seemed attractive in view of the menace of atomic warfare, which was at that time even more of a reality than it is today.

HUMORAL HYPOTHESIS

The humoral hypothesis received what seemed to be strong direct support from the work of Cole's group at San Francisco[82, 83, 85, 196]. These workers found that cell-free homogenates of spleen and bone marrow were therapeutically effective and they claimed that this

could not be explained by the presence of intact cells in their preparations. On the basis of studies involving the fractionation of spleen "homogenates" and the "specific" destruction of cell nucleii in the "homogenates", Cole *et al.* postulated:

(a) "That the cell nuclei of normal mouse spleen contain a specific macromolecular nucleoprotein complex which is required for the cell growth and division, regeneration or maturation of critical haemopoietic tissue"; and

(b) "that the biological activity of this nucleoprotein is inhibited by ionizing radiations"[85].

Other workers were, however, unable to confirm these results[21, 190] and it must now be concluded that the findings of Cole *et al.* were due to the fact that these "homogenates" contained large numbers of intact viable cells. It should be mentioned that the same group was among the first to publish experimental proof for the cellular mechanism in 1956.

Indirect evidence which seemed to support the humoral hypothesis came from two sets of observations by Jacobson and his coworkers.

(1) Survival following irradiation and spleen implantation was not always accompanied by the persistence of the spleen graft[187]. However, this observation contradicted earlier reports by the same author and it was not subsequently confirmed by others. When one of the present authors reinvestigated this important point in 1954[47], a strong correlation was found between the survival of the irradiated mice and a take of the (isologous) spleen grafts.

(2) The degree of protection afforded by spleen shielding was not modified when the shielded spleen was removed completely 1 hour after the end of the irradiation[186]. Even when the splenectomy was performed five minutes after the irradiation some significant protection remained. These results are now explained by the fact that repopulation of the destroyed haemopoietic tissues can be accomplished by the seeding of an amazingly small number of (isologous or autologous) haemopoietic cells, but at that time the results seemed to fit the humoral hypothesis far better.

Perhaps the most forceful argument against the cellular mechanism was provided by the therapeutic successes which Lorenz *et al.*[98, 230] obtained with homologous and heterologous tissues. At that time, the

idea of an heterologous transplant becoming established was completely at variance with the current biological dogmas. Evidently, it was not sufficiently recognised that the immunological defence reactions are very severely inhibited following lethal doses of radiation, so as to permit the establishment of a foreign graft.

Even those investigators who favoured the cellular hypothesis were initially prepared to accept the findings of Lorenz *et al.* as a seemingly insurmountable obstacle. To quote from a paper by Barnes and Loutit[28] in 1954: "One cannot presume that heterospecific (i.e. guinea-pig to mouse) cellular material would survive in the host. . . . Therefore this indirect approach (of Lorenz) suggested that cells that were doomed to die, were potent, presumably in virtue of their supplying a chemical not a vital factor."*

In order to test such a possibility an attempt was made to supply this chemical factor regularly by the daily administration of large amounts of proven *cell free* spleen extract to irradiated mice for the period of a week following the irradiation. This treatment failed to reduce the mortality[118].

CELLULAR HYPOTHESIS

Barnes and Loutit, among others, were unable to confirm the therapeutic effectiveness of heterologous bone marrow as described by Lorenz *et al.* In addition, they and other workers made a variety of observations which appeared to be far better explained by the cellular than by the humoral mechanism. The observations in themselves constituted, however, no more than circumstantial evidence.

These results may be summarised as follows:

(1) The intravenous administration of haemopoietic cell suspensions proved to be far superior to an intraperitoneal injection.

(2) Any procedure tested which would tend to decrease the viability of living cells (e.g. heat, ionising radiation, freezing) was found to decrease the effectiveness of the preparation. Conversely, storage of the factor for appreciable periods in the frozen state was found to be possible only when methods were used that were known to favour the preservation of living cells[30].

(3) Mice treated with homologous spleen, although able to survive the 30th day after irradiation, had all died by day 100, in contrast to mice receiving isologous spleen, which lived for 600

* In the same paper these authors concluded that "the chemical hypothesis has not been proved by the complete exclusion of the cellular hypothesis".

days and longer[29]. Such a delayed difference between the action of homologous and isologous material seemed more in accordance with a tissue grafting mechanism than with the effects to be expected from a humoral factor.

This striking difference between the action of isologous and homologous spleen had escaped the attention of Lorenz *et al.* because they employed a relatively short observation period of 20–30 days.

(4) When recipient mice were immunised against the donor strain prior to the irradiation, the administration of homologous spleen no longer resulted in a prolongation of survival time[28]. This finding could be explained by assuming that the humoral factor possessed antigenic properties, but again the cellular mechanism seemed to offer a more likely explanation.

(5) Finally, certain observations of Main and Prehn[239] very strongly suggested the replacement of host haemopoiesis by the donor type cells. These authors transplanted BALB skin to DBA mice which had been treated with (BALB × DBA)F_1 bone marrow following irradiation. The skin grafts took in the majority of the cases (see Fig. I[1]).

Shortly afterwards, Lindsley *et al.*[222] supplied direct evidence that transplantation and proliferation of haemopoietic cells can be achieved in irradiated rats under certain conditions. By the use of a serological method, they were able to demonstrate the presence of donor type erythrocytes several months after the irradiation and the bone marrow grafting.

In a limited number of cases an almost complete replacement of host erythrocytes had occurred but in others the majority of the erythrocytes were of host type. Although these results were highly significant they failed to prove satisfactorily the validity of the cellular mechanism; firstly, because the results failed to show a strong correlation between survival of the irradiated rats and the occurrence of replacement of host type by donor type erythropoiesis, and secondly, because the donor and recipient strains were genetically quite closely related (as evidenced by the subsequent observation that 20–25 per cent of permanent bone marrow transplants could be achieved following sublethal doses of X-rays)[295]. The occurrence of donor type erythropoiesis could, therefore, have been independent of the curative effect of the bone marrow.

From the nature of the various experiments described above it

can be deduced that by the end of 1955 there was a general tendency to abandon the humoral factor hypothesis in favour of the cellular mechanism. In fact a number of investigators had already set out to devise ways and means to settle this question.

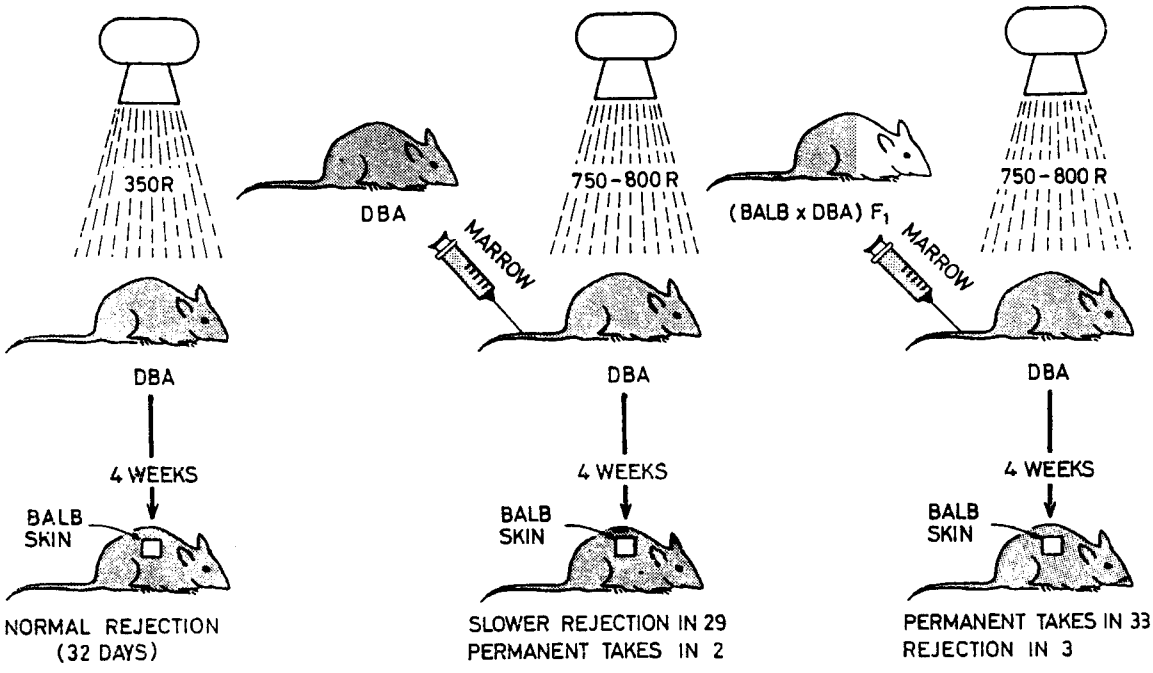


Figure I[1]. Schematic representation of the results obtained by Main and Prehn (1955)[239]

From left to right

Sublethally irradiated DBA mice reject a BALB skin graft normally.
Lethally irradiated DBA mice treated with isologous bone marrow show delayed rejection of BALB skin grafts.
Lethally irradiated DBA mice treated with (BALB × DBA)F_1 bone marrow accept BALB skin grafts in the majority of cases.

IDENTIFICATION OF GRAFTED CELLS

In 1956 three research teams independently succeeded in supplying irrefutable proof of the cellular mechanism. That the three papers were published simultaneously—in March—in three different journals must be interpreted as pure coincidence, considering the procedures involved nowadays in publishing scientific papers. Surprisingly, there was little duplication in the methods employed in the three laboratories.

A very elegant technique for the identification of donor type cells in the mice treated with bone marrow was introduced by Ford *et al.*[143]. These workers were able to prepare chromosomal preparations of the haemopoietic cells in metaphase. Rat cells contain 42 pairs of chromo-

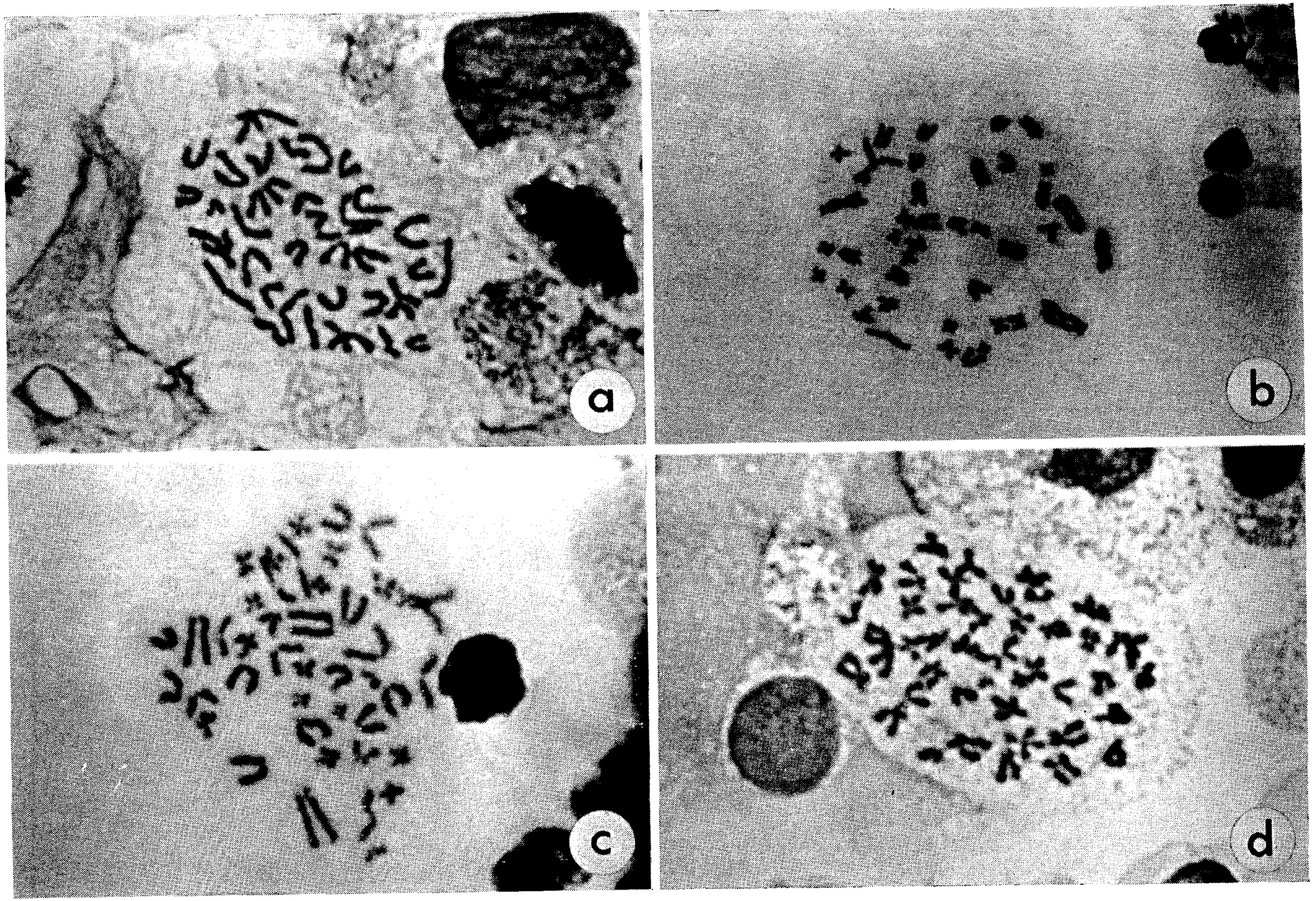
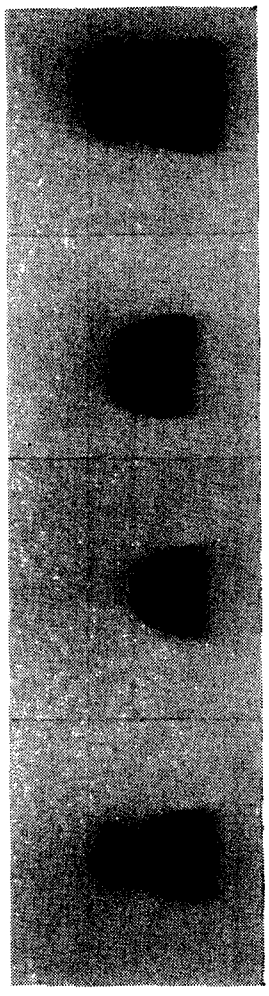


PLATE I:1. Squash preparations of cells from the bone marrow showing the chromosomes in metaphase
(a) Mouse (b) Rat (c) Mouse irradiated and treated with rat bone marrow 21 days previously
(d) Mouse irradiated and treated with bone marrow from a Syrian hamster 79 days previously



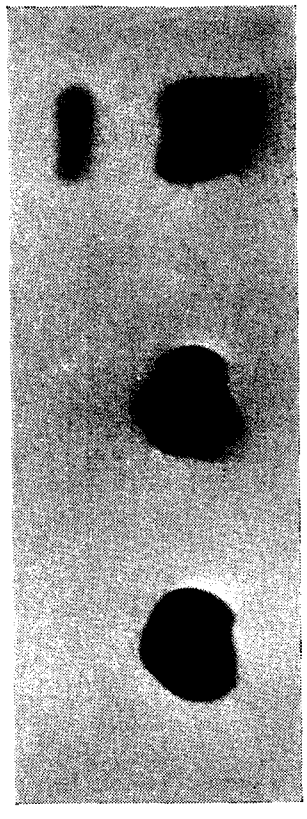
CBA MOUSE

C_{57}BL MOUSE

CHIMAERA: CBA + C_{57}BL BONE MARROW

CHIMAERA: C_{57}BL + CBA BONE MARROW

a



$(RF \times CBA)F_1$ MOUSE

SYRIAN HAMSTER

CHIMAERA: $(RF \times CBA)F_1$ MOUSE + HAMSTER BONE MARROW

b

(Courtesy Dr. H. M. Klouwen, Rijswijk)

PLATE I:2(a) and (b). Agar electrophoretograms of haemoglobins of various mouse strains, Syrian hamsters and mouse radiation chimaeras

(a) chimaeras 5 and 9 months after bone marrow transplantation
(b) chimaera 3 months after bone marrow transplantation

somes and a number of them have a characteristic cruciform (metacentric) appearance in metaphase. The mouse cell has 40 chromosome pairs all with terminal centromeres. It is easy to distinguish, therefore, rat and mouse cells (Plate I: 1) in metaphase, and when this method was applied to haemopoietic cell preparations of mice treated with rat bone marrow, the majority of the cells was found to contain the rat-type chromosomes.

In addition, the group at Harwell employed donor mice which carried a clearly recognisable translocation on one of the chromosomes (T_6 mice) and they were able to show the presence of the marker chromosome in metaphase haemopoietic cells of the treated recipients. The authors correctly concluded that it is inconceivable that the host cells had accepted whole chromosomes from the donor cells, rejected some of their own to maintain a normal complement and still retained a balanced set for division. A transformation of host cell chromosomes through the influence of the injected foreign material was equally inconceivable, and these findings provided, therefore, indisputable proof of a cellular repopulation mechanism.

Donor type cells were identified with this technique not only in the bone marrow but also in the spleen, the lymph nodes and the thymus. Elegant and conclusive as this method is, it also possesses definite disadvantages for routine studies of this kind. The applicability is limited because the animal has to be either sacrificed or operated upon. Furthermore, only a small proportion of the cells in the tissues to be examined presents its chromosomes in the squash preparation in such a way that identification becomes possible. Finally, a cytological classification of the haemopoietic cells in metaphase is very difficult indeed.

The Rijswijk group provided proof of the cellular hypothesis in three different ways[438]. Two methods were at that time used for the rat–mouse chimaera. The first consisted of a serological identification of the erythrocytes with the use of specific agglutinating antisera. A slightly positive reaction with anti-rat serum was obtained as early as 10 days following rat bone marrow transplantation and the complete replacement of mouse erythrocytes by rat erythrocytes had occurred at about 60 days.

The mere demonstration of erythrocytes which agglutinated in the presence of anti-rat serum did not constitute a wholly satisfactory proof of the cellular mechanism since the possibility that a number of mouse erythrocytes had collected rat proteins on their outer surface

could not be excluded. The observation, however, that the proportion of cells reacting with anti-rat serum increased in the course of about 2 months following transplantation to reach 100 per cent, while simultaneously the proportion of cells reacting with anti-mouse sera decreased, was less subject to such criticism.

The method described requires only a few drops of blood from the tail and a single animal can be tested repeatedly for any period of time. The identification is limited, of course, to the erythropoietic system.

The second technique which was used permitted typing of the myelopoietic system only. It was a histochemical assay based on the observation by Wachstein[452] that rat granulocytes yield a strongly positive alkaline phosphatase reaction, while mouse granulocytes are essentially negative in this respect. After treatment with rat bone marrow, positive cells appeared in the blood of irradiated mice within a few days and after about 8 days the majority of the cells showed a positive reaction (see colour frontispiece). It could be argued, of course, that the injection of rat material might have induced alkaline phosphatase activity in mouse granulocytes but this type of reasoning is extremely far-fetched. However, some caution in an interpretation of these results was clearly necessary, as the authors pointed out: "Although the histo-chemical evidence as such cannot be accepted as unequivocal proof of the cellular hypothesis, it constitutes a substantial support for the two other arguments presented in this paper."

The same method was employed at San Francisco by Nowell *et al.*[293] who also found a replacement of alkaline phosphatase negative granulocytes by positive cells following the transplantation of rat bone marrow into irradiated mice. In addition, they observed a tremendous proliferation of positive cells in the bone marrow in the first weeks following transplantation.*

This method of distinguishing rat and mouse granulocytes has proved to be extremely suitable for the repeated typing of individual animals. The test can be performed with a small drop of tail blood and the differentiation of hundreds of cells can be carried out both

* Recent observations from the authors' laboratory indicate that the percentage of alkaline phosphatase positive cells may vary somewhat between different rat strains. However, in all the strains so far investigated the majority of the granulocytes have been positive. Since in germfree and mono-contaminated gnotobiotic rats the granulocytes were found to be alkaline positive, there seems to be no relationship between the presence of infections and the alkaline phosphatase of the granulocytes in this species, in contrast to the findings in humans reported by Wachstein.

rapidly and reproducibly. Since control rat blood always yields more than 95 % positive granulocytes, for all practical purposes the finding of more than 5 % negative cells can be interpreted as evidence of the presence of mouse granulocytes.

The third procedure which was employed by Davids *et al.* to investigate the cellular hypothesis can best be described as a typing of the whole population of haemopoietic cells in the bone marrow. It was based on the observation by the same authors that the number of homologous (and heterologous) bone marrow cells required to prevent mortality in lethally irradiated mice is about 20 times larger than the number of isologous cells necessary to achieve a similar therapeutic effect.

The identity of the bone marrow of mice which survive the irradiation as a result of homologous bone marrow injection could be established, therefore, by the injection of graded numbers of these bone marrow cells into two strains of irradiated mice, one identical to the recipient and the other identical to the original bone marrow donor. This assay is depicted in Fig. I[2]. The results left no doubt that the majority of the bone marrow cells in the surviving recipients were of donor origin.

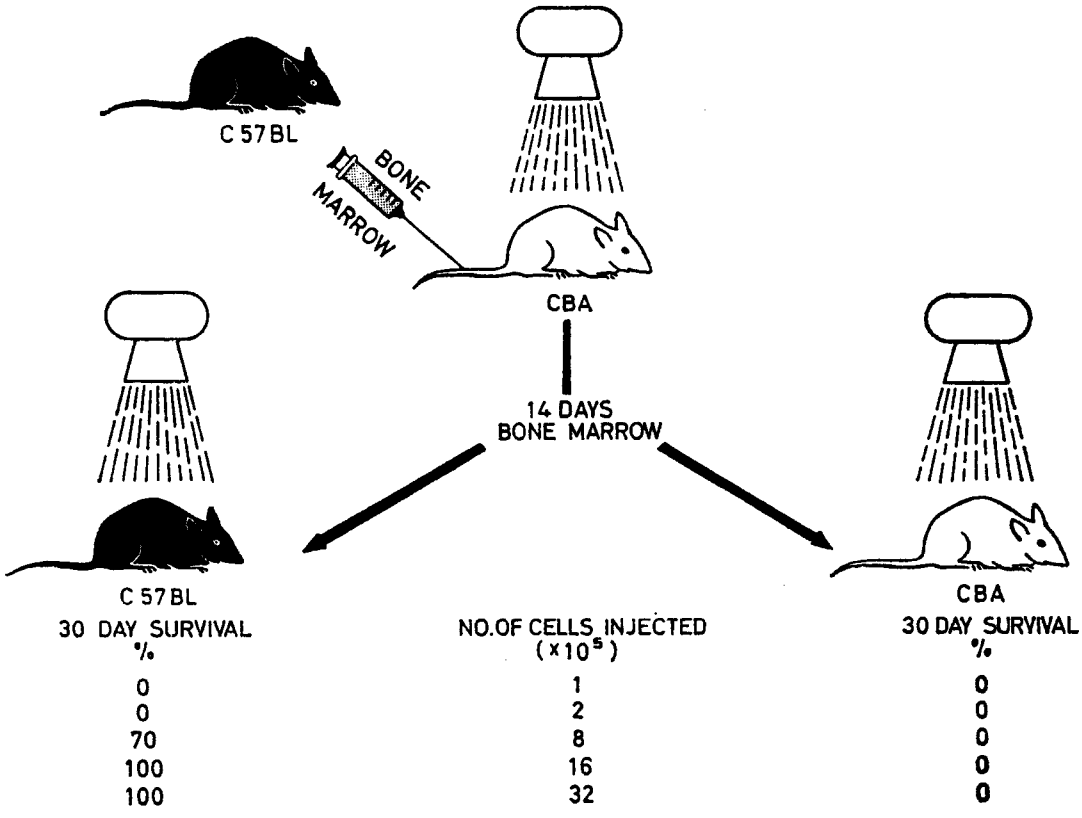
Thus, the publications which appeared in March 1956 provided independent evidence in favour of the cellular mechanism by four different procedures. These findings fully justified the acceptance of the earlier observation by Lindsley *et al.* as having established a similar state of events in their rats treated with homologous bone marrow.

A great number of other publications containing confirmatory, as well as additional evidence have subsequently appeared. Methods have been successfully worked out to identify all types of cells which have their origin in the haemopoietic system and a number of other host donor combinations have been investigated with similar results.

It can now be concluded with certainty, therefore, that under suitable conditions the host's haemopoietic system may be replaced by isologous, homologous and heterologous cells according to the type of bone marrow donor.

A list of the identification methods which are presently available is presented in Table I: 3. Many can be made to yield quantitative or semi-quantitative results of the proportion of host and donor type cells in the test sample. This holds in general for the serological assays and for the histochemical test. The electrophoretic method

for the identification of haemoglobins in suitable host donor combinations produces at best semi-quantitative results (Plate I: 2) and the drum stick counts in female–male combinations can give qualitative information only (Plate I: 3)[237].



C 57 BL 30 DAY SURVIVAL %	NO. OF CELLS INJECTED ($\times 10^5$)	CBA 30 DAY SURVIVAL %
0	1	0
0	2	0
70	8	0
100	16	0
100	32	0

Figure I[2]. Transplantation of bone marrow from a radiation chimaera as a method of identification of the cells

The bone marrow cells of lethally irradiated CBA mice which were treated with C57BL bone marrow were able to protect lethally irradiated C57BL mice but not lethally irradiated CBA mice, when injected in moderate numbers. Since protection with such small amounts of bone marrow is only possible in isologous host-donor combinations, this is proof that the original CBA recipient carried C57BL cells in its bone marrow at the time of the identification experiment. (van Bekkum *et al.*, 1956)[51]

When bone marrow transplantation has been performed in human patients, it has usually been possible to employ differences in one or more minor blood groups between the donor and the recipient, so that a convenient differentiation of erythrocytes can be made. Differences in the leucocyte antigens between the donor and the recipient can be employed in principle, but as yet the application of this technique is limited to primates[117, 472] and dogs[473], for which the iso-

antisera available allow the identification of the "antigenic profile" of a leucocyte sample. To trace the myelopoietic cells of the donor in human patients, it would also be useful if the donor could be selected from the opposite sex as the recipient, in order that the drum stick count of the granulocytes could be followed.*

Summarising, the identification methods fall into four classes: cytological, serological, biological (serial transplantation) and biochemical (haemoglobin electrophoresis).

Immunological specificity of chimaeras

Attempts at identification based on the capacity to produce specific antibodies have not been listed in Table I: 3 because the evidence obtained by this approach is usually rather indirect and not sufficient in itself. Nevertheless, useful information in favour of the cellular hypothesis has been obtained by such experiments. The first attempt in this direction was made by Mitchison[283] who showed that irradiated mice treated with spleen cells of mice immunised with *Salmonella typhi* (H) antigen, showed a greater production of antibody than normal mice receiving the same treatment.

In irradiated CBA mice, Mitchison[283] further demonstrated the increase of A isoantigens and their persistence for at least 51 days following A spleen cell transplantation. The chimaeric tissues to be tested were injected into normal CBA mice which were challenged subsequently with an A tumour. Growth inhibition of the tumour was considered as evidence of the presence of A antigens in the tissue sample. A comparable method, which employed the disintegration of a non-vascularised Harderian gland homograft in pre-immunised mice, was reported by Merwin and Congdon[275].

The results of these two investigations were similar, in that evidence of the presence of donor type antigens was found in a number of haemopoietic tissues of the treated mice, including the lymph nodes and the thymus. It was later confirmed with more direct methods (see Table I: 3) that complete repopulation of these organs with donor type cells might occur; this means that in established radiation chimaeras the host's lymphatic tissue has been replaced by a population of donor derived lymphoid cells. Since the lymphoid system is the site of immunological reactivity, it follows that the immunological

* In the case of a male recipient this procedure theoretically carries an increased risk of graft versus host reactions, because the presence of sex-linked histocompatability genes has not been excluded in humans.

TABLE I: 3. Methods of differentiation between host and donor derived cells in radiation chimaeras

Cell type	Host-Donor combination	Method	Authors
Haemopoietic cells in general	Mouse–mouse Mouse–rat Mouse–hamster	Chromosome identification	Ford *et al.* (1956)[143] Ford *et al.* (1956)[143] van Bekkum (1964)[44]
Bone marrow	Mouse–mouse	Serial transplantation	Vos *et al.* (1956)[438]
Erythrocytes	Rat–rat	Agglutination with specific antisera	Lindsley *et al.* (1955)[222]
	Mouse–rat	Agglutination with specific antisera	Vos *et al.* (1956)[438] Makinodan (1956)[241]
	Mouse–hamster	Agglutination with specific antisera	van Bekkum (1964)[44]
	Pigeon–pigeon	Agglutination with specific antisera	Shaw and Vermund (1959)[366]
	Pigeon–pigeon	Agglutination with *Phaseolus limensis* extracts	Shaw and Vermund (1959)[366]
	Pigeon–dove	Agglutination with specific antisera	Shaw and Vermund (1959)[366]
	Monkey–monkey	Modified Coomb's reaction	Owen (1961)[301]
	Mouse–mouse	Electrophoresis of haemoglobin	Rosa *et al.* (1958)[353] Welling and van Bekkum (1958)[453] Popp *et al.* (1958)[319]
	Monkey–monkey (Rhesus) (Cynomolgus)	Solubility of haemoglobin	Overman (1959)[298]
	Mouse–mouse	Solubility of haemoglobin	Popp and Cosgrove (1959)[318]

Granulocytes	Mouse–rat	Alkaline phosphatase	Nowell *et al.* (1956)[293] Vos *et al.* (1956)[438]
	Mouse–hamster	Alkaline phosphatase	van Bekkum (1964)[44]
	Rabbit–rabbit	Pelger anomaly in donors	Czerski *et al.* (1960)[115]
	Male–female rabbits	Drumstick appendages	Porter (1957)[323]
	Male–female dogs	Drumstick appendages	Porter and Couch (1959)[330] Mannick *et al.* (1959)[248]
	Male–female monkeys	Drumstick appendages	Magliulo *et al.* (1963)[237]
Thrombocytes	Mouse–rat	Agglutination with specific antisera	Smith *et al.* (1957)[380]
Thymus cells	Mouse–rat	Agglutination with specific antisera	Urso *et al.* (1957)[431]
	Mouse–rat	Chromosome identification	Ford *et al.* (1956)[143]
	Mouse– mouse (T 6)		
	Mouse–mouse	Graft versus host test	Popp (1961)[317]
Lymph node cells	Mouse–rat	Chromosome identification	Ford et al. (1956)[143]
	Mouse–mouse (T 6)		
	Mouse–rat	Cytotoxic test with antisera	Zaalberg and van Bekkum (1959)[469]
	Mouse–rat	Tissue globulin electrophoresis	Popp and Smith (1959)[321]
	Mouse–mouse	Cytotoxic test with antisera	Vos *et al.* (1960)[439]
Macrophages (peritoneal)	Mouse–mouse	Cytotoxic test with antisera	Balner (1963)[13]

reactivity of radiation chimaeras should have all the characteristics of that of the donor.

The skin transplantation experiments of Main and Prehn, which have been mentioned earlier, were the first to focus attention on this possibility. These findings were confirmed and extended soon afterwards by Trentin[414] and by Zaalberg *et al.*[470]. The latter workers demonstrated for the first time that rat skin can be made to grow on a mouse when the recipient is pretreated with total body irradiation and rat bone marrow transplantation.

Spontaneous blood chimaerism, a comparable but not identical condition, occurs naturally in some dizygotic twins of certain species (cattle[299], sheep[389], marmoset[55] and man[351, 465]) and is caused by exchange of haemopoietic cells in foetal life between the twin partners through vascular anastomoses. This results in the establishment of a mixed population of haemopoietic cells which persist in one or both partners into adult life. Anderson *et al.*[6] introduced the term genetical chimaera for this condition. It has been experimentally produced in chickens by artificial union of the chorioallantoic membranes of two embryos by Hasek[169]. Some authors have used the term blood or marrow mosaicism, but recently chimaerism seems to be the more generally accepted term.

It is already evident that the implications of these discoveries reach far beyond the treatment of radiation sickness. The homologous and even the heterologous transplantation of an extremely complex tissue, with localisations throughout the entire host organism has been shown to be possible by the simple procedure of an intravenous injection of a limited number of cells. In many cases this complex transplant remains permanently established with preservation of its various specialised functions, while the analogous cells of host origin do not return. Recently, it has even become apparent that macrophages as they occur in the peritoneal cavity, will be replaced by donor type cells in radiation chimaeras[13], but the fate of the so-called fixed macrophages still has to be ascertained.

Investigators in the fields of transplantation biology, immunology and haematology have been quick to recognise the unique possibilities of the radiation chimaera as an experimental tool.

In the following chapters many aspects of radiation chimaeras will be discussed. A wealth of experimental data is now available and many of the essential questions concerned with chimaerism have been answered. In dealing with these problems the authors have referred

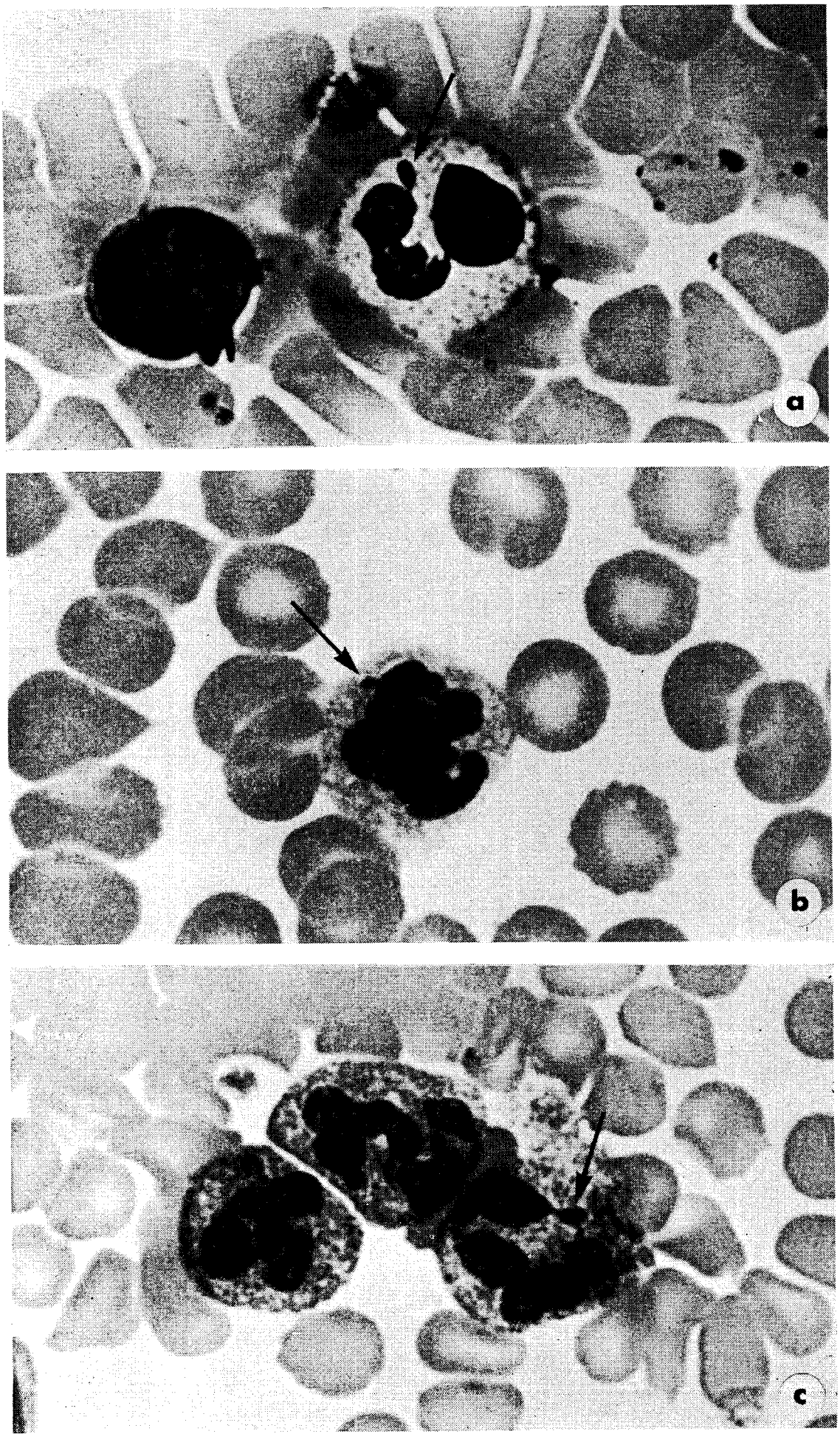


PLATE I: 3. Characteristic drumstick appendages in neutrophil granulocytes

(a) human female (b) rhesus monkey female (c) rabbit female

primarily to those papers which provide clearly interpretable results. The merit of such experiments can be evaluated properly only if information is available to prove the chimaeric state of the animals. It is a mistake to assume that the injection of an arbitrary number of bone marrow cells into an animal irradiated with a supposedly lethal dose of X-rays will always produce permanent haemopoietic chimaerism. These factors can be and have to be controlled. Since 1956 and 1957 a sufficiently large number of comparatively simple methods has been available to obtain exact information on the state of chimaerism of the experimental animals at any time. Studies in which this condition is not fulfilled have been intentionally neglected in this review in favour of those which meet this criterion even if the latter have been published at a later date.