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J. G. BUGHER
The Rockefeller Foundation, New York

J. COURSAGET
C.E.A., Saclay

J. F. LOUTIT
Medical Research Council, A.E.R.E., Harwell

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EDITORIAL INTRODUCTION

THE Editors of this volume have never been editors of a great journal such as *The Times*. Nevertheless in some ways their problems are similar. A large number of news items drawn from sources all over the world have been received at one point in time. Some are of limited or local interest only: some are of a general and fundamental character but their importance is particularly relative to their time: some will make history. The unhappy editor of *The Times* will reject what he feels is unreliable but he has to include in his daily edition something of each of the above categories. On the other hand he can prune and shape his material according to his needs and views and he enjoys the wonderful prerogative of weighting for his readers the various contributions by the size of his headlines.

The editors of this volume fortunately have not the task of performing their duties every day, but unlike the editor of secular news they cannot reject scientific news on an impression that it may not be the truth and nothing but the truth. (It is too much to hope for in science that in one communication one will get the whole truth.) They have chosen therefore from papers presented to the 2nd International Conference on the Peaceful Uses of Atomic Energy those in the biological fields which appealed to them as representative of local interest in some part of the world, of general interest at the present time or of permanent historical value. They have made no cuts, so that apart from a few minor editorial corrections, the papers are exactly as their authors thought most fit; and they have made no attempt to bias the readers of this volume by editorial opinion in any form. The material which the editors consider will "make history" are not to be determined by what in the jargon of the geneticist might be called "position-effects".

On the occasion of the 1st Conference at Geneva in 1955, the volume, "Biological Sciences" in the *Progress of Nuclear Energy* Series reported the biological contributions to the conference in two ways. There were as in this present volume exact reproductions of the original papers and also reviews of special subjects of particular interest at the conference. There is no doubt that reviews can be invaluable both to the tyro seeking for knowledge and to the established savant in other fields, but perhaps their greatest benefit is to the writer, already an acknowledged expert in his field, because this exercise permits him to survey and evaluate his own field. On the other hand even for the acknowledged expert it takes much time and toil to produce the critical review of which he can be justly proud. In 1958, therefore, it was an editorial decision to give all the weight to the contemporary thought of the many with its quicker publication, and none to the critical evaluation of the few.

This decision, which may well have been the wrong one, identified one of the major problems confronting the cultivated and learned of today and especially tomorrow. As a result of research and development observations are being made in limited fields at a rate too great even for the genius to absorb and

digest. Thus we have to envisage that in the not too distant future the genius, and the ordinary man too, will need his information presented so that nothing of the past which is relevant or valuable is lost and forgotten. To implement this further, developments in automatic methods are needed. However unpleasant the prospect may be it is necessary to consider also whether the critical synthesis should not also be made by automation.

J. F. LOUTIT

PUBLISHERS' NOTE

The Geneva Conference (1958) paper number of each article is given as a footnote on the first page. While these articles are in general in the form in which they were submitted to the Conference, authors have been given an opportunity to make revisions and corrections and many have made substantial alterations to their original contributions. The Editors and the Publishers would like to express their thanks to all authors for their helpful co-operation with the publication of this volume.

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SPONTANEOUS AND INDUCED CHANGES IN CELL POPULATIONS IN HEAVILY IRRADIATED MICE

By D. W. H. BARNES, C. E. FORD, S. M. GRAY and J. F. LOUTIT

Medical Research Council Radiobiological Research Unit, A.E.R.E., Harwell, Berkshire, England

INTRODUCTION

1. At the last Conference on the Peaceful Uses of Atomic Energy we [1] concluded a discussion on recovery of the lethally irradiated mouse following treatment with biological materials (such as bone marrow and spleen) with the words, "Until proven cell-free materials are shown to be active, we continue to assume that the stimulation of the processes of recovery is accomplished by living cells". Hitherto it had been argued that recovery was dependent on humoral agents in the material administered as treatment. This forecast has been confirmed in the intervening years in many laboratories [2–10] by immunological, histochemical and cytological methods. The recovered animal is thus a chimaera containing populations of cells derived both from the original host and the donated material.

2. The chimaera may be physiologically stable. For instance, in this laboratory, the subsequent life of the 3-months-old CBA male mouse irradiated with 950 rads of X-rays and treated with spleen from infant mice of the same in-bred strain (isologous chimaera) was 500 days median [11]. On the other hand, similar CBA irradiated mice treated with material from other strains of mouse (homologous chimaera) or from rats (heterologous chimaera) survive for variable periods. Many die within 3 to 4 months [11, 12, 13] from a "secondary disease" with loss of weight and general condition, diarrhoea and perhaps dermatitis. This secondary phenomenon has been attributed to be an immunological reaction between the two populations of cells.

3. In addition to this instability of function—and as far as we can ascertain independent of it—there may be instability of the cell-populations relative to each other in the myeloid and lymphoid tissues. In previous communications [14, 15] we have noted that, in bone marrow and spleen up to about a week after irradiation and in lymphoid tissue for about 3 weeks, dividing cells of the *host* animal may be found which exhibit extensive breakage and rearrangement of their chromosomes. Then follows a period during which normal dividing cells of the donor's type only are found. This state may persist throughout the whole of the chimaera's subsequent life, as was the case in the CBA/T6 homologous chimaera: alternatively in some combinations of host and donor after a variable period native cells reappear and partially or wholly replace the donor's cells, a condition that we call "spontaneous reversion".

Geneva Conference Paper (1958) P/97

4. In this paper we report the detailed cytology of these reverted tissues showing that in some cases there are chromosomal rearrangements. It is perhaps not surprising that some of the regenerated cells of the host should reveal their history of exposure to radiation in this way. What *is* notable is that precisely the same pattern of rearrangement should be observed in many different cells of several different organs. In view of the known randomness of chromosome-breakage and the great variety of changes of this kind, the conclusion is that all the cells exhibiting the same set of chromosomal rearrangements are related by mitotic descent from a single ancestral cell which was changed as a result of the irradiation. In other words, they constitute a clone of cells *in vivo* [15]. It should be added that the chromosomal changes found in these cells are *balanced:* there is no evidence of either loss or gain of chromosomal material and the chromosome number remains constantly 40. This situation is to be compared, on the one hand, with that in corresponding normal tissues—where the evidence points to almost complete constancy of chromosome-form as well as number [16]—and contrasted, on the other hand, with the appearance of chromosomes in neoplasms of the reticulo-endothelial system, in many of which *unbalanced* changes of both form and number are present, some of them highly specific to the individual neoplasm [17].

5. This paper also reports preliminary data on "induced transpopulation" in the homologous chimaera. The chimaera CBA/T6×CBA which should be even more stable than that formerly reported—CBA/T6+ (in fact CBA/T6×3HI *vide infra*) was further treated two weeks after its production with suspensions of isologous tissues (CBA spleen or lymph node). Change to normal chromosomal pattern, i.e. CBA, was induced by the suspension of spleen, but not in the doses given by lymph node.

METHODS AND MATERIALS

6. *Mice*—The CBA/H mice, the irradiated hosts, in all but one instance, and the C57/H mice, the one exception, were the Harwell strains of the well-known CBA wild-type and C57 black, in-bred lines. The stocks have been maintained by our geneticists (Carter, Lyon and Phillips) by strict sib-mating with frequent re-selection of lines.

7. The murine donors of myeloid tissue were hybrids. Mice of the T6 stock of Carter, Lyon and Phillips [18] have a radiation-induced chromosome-translocation which is cytologically identifiable at the metaphase of mitosis, one of the chromosomes being about half the length of the smallest chromosome seen in normal mouse cells [4]. Homozygous T6.T6 male mice, which are genetically heterogeneous, are mated with CBA females to produce the $(CBA \times T6)F_1$. They have also been mated with $(C3H \times 101)F_1$ hybrids to give what in previous communications [4, 14, 15] were called T6/+ mice and now called (3HI×T6).

8. *Rats*—The rats from which myeloid tissue has been taken are albino and are reported to stem from Wistar stock. They also have been sib-mated for many generations by our geneticists.

9. *X-radiation*—The irradiation of the mice was carried out by the standard practice of the laboratory [19]. The dose of X-rays (240kV.c.p.; 15 mA; HVL

1·2 mm Cu at 43r/min) was calculated to be 800 or 850 or 950 rads according to the experiment and the strain of mice irradiated.

10. *Preparation of suspensions of bone marrow*—The two femora from one rat or from one mouse provided the suspension of cells used for the recolonization of 5 irradiated mice. The epiphyses were avulsed from the excised bones and the marrow from the shafts of the bone was blown by compressed air into a solution of 0·9% NaCl. Equal volumes, 0·2 or 0·4 ml., of the suspension were injected intravenously into each recipient, which received about 2 to 4×10^6 nucleated cells when the donor was the mouse or 2 to 4×10^7 cells with the rat as donor.

11. *Preparation of suspensions of spleen or lymph gland*—The spleen was excised from a freshly sacrificed CBA/H mouse, suspended in normal saline or rabbit serum, and shredded by means of an electrically-operated mincer; after the fibrous stroma had settled by gravity the supernatant cell-suspension was sucked into an all-glass syringe and used immediately for injection.

12. The peripheral lymph nodes (inguinal, axillary and brachial) were excised and a suspension of cells prepared in similar fashion. The quantities of spleen and lymph node used per mouse are given in Table I.

13. Rabbit serum was used on some occasions rather than saline as a vehicle: it allows better dispersion of the material so that reactions, including deaths due to embolism, are lessened. The heterologous serum seems to have no ill-effect—an observation confirmed by Makinodan [20].

14. *Sensitization of CBA/H mice*—When a (CBA×T6) mouse was sacrificed as a donor for bone marrow, its spleen was excised, minced in saline and injected intraperitoneally into a CBA/H mouse, which two weeks later was sacrificed to provide sensitized spleen or sensitized lymph nodes.

TABLE I

Amounts of Spleen or Lymph Nodes given per Mouse in Attempts to Induce Transpopulation

Experiment No.	Series (a) Volume of vehicle only injected	Series (b) Mass of normal tissue used per mouse and volume of vehicle	Series (c) Mass of sensitized tissue used per mouse and volume of vehicle
I	0·4 ml. saline	24 mg spleen in 0·4 ml. saline	26 mg spleen in 0·4 ml. saline
II	0·3 ml. serum-saline	7 mg spleen in 0·3 ml. serum-saline	23 mg spleen in 0·4 ml. saline
III	0·4 ml. saline	8 mg. lymph node in 0·4 ml. serum	10 mg lymph node in 0·4 ml. serum

15. *Cytological methods*—The mice were injected between 1½ and 2 hours before sacrifice with 0·01 ml. of 0·04% solution of Colcemid per gram of body weight. The bone marrow from the femoral shafts, pieces of spleen, the thymus

and one or more peripheral lymph nodes were taken and suspended or chopped up in a warm *hypotonic* solution of sodium citrate (1·12% w/v). They were then fixed for 1 to 2 hours in acetic alcohol (1 part glacial acetic acid to 3 parts of absolute ethyl alcohol) before being hydrolysed in normal hydrochloric acid and stained with Feulgen reagent. The suspended bone marrow cells had to be spun down between transfers; the chopped "solid" tissue-fragments settled by gravity and exchanges were effected by decanting. Permanent squash preparations were made. A more detailed account of the method has been given elsewhere [21]. The preparations from which the data of Table III were obtained were randomized and scored as unknowns.

RESULTS

16. *Identification of clones in "spontaneous reversion"*—The first example of the phenomenon was discovered in a CBA mouse (M1025) which had been irradiated (850r) and injected with rat bone marrow 77 days before it was sacrificed. In this animal a clone of cells, characterized by one exceptionally long chromosome and one no less exceptionally short, was present in bone marrow, spleen and the two inguinal lymph nodes, reaching a level of 32% of the dividing cells in the latter organs. This case and another (M549) have already been reported briefly [15]. Altogether, the tissues of 28 chimaeras, in which there had been partial or complete regeneration of host-type tissue, have been examined for clones. "Good" clones (i.e. clones with readily detectable chromosome changes) were found in 8 of them and "probable" clones in a further 12. It was, of course, to be expected that where chromosome rearrangements occur they would vary from the highly individual and striking to the barely detectable. It was also to be expected that in some animals more than one clone would be found, and this, in fact, has been observed in several instances.

17. Similar cell-clones have been recorded in another combination, that of C57BL host and 3HI×T6 donor. Of 3 animals in which there had been recovery of host cells "good" clones were found in 2. One of these clones proved to be the most remarkable yet seen. It was marked by a long metacentric chromosome and another (normal acrocentric) which was exceptionally small. Of the dividing cells examined, 95% in bone marrow, 55% in spleen, 80% in thymus and 67% in lymph node were all of this type.

18. Estimates of the extent to which a given clone has multiplied in a particular organ in tissue can only be based on the proportion of the dividing cells which exhibit change. For an accurate assessment a well-marked clone and good preparations are equally necessary. The results obtained in 5 cases where both these conditions were met are given in Table II. Figs. 1 and 2 are photographs of two of the clonal types.

ESSAYS IN "INDUCED TRANSPOPULATION" OF CBA/CBA×T6 CHIMAERAS WITH SPLEEN AND LYMPH NODE

19. Homologous chimaeras were produced by irradiation of CBA mice with 950 rads of X-rays followed by intravenous injection of (CBA×T6)F_1 bone marrow. After two weeks, having recovered from the immediate effects of the

radiation syndrome, they were divided into 3 groups and were injected intravenously with

(a) saline solution—mock treatment
(b) suspension of normal spleen-cells
(c) suspension of sensitized spleen-cells.

TABLE II

Cell-clones in Regenerated Host Tissue

Mouse No.	Dose of X-rays delivered	Host mouse	Donor type	Days from irradiation to sacrifice	Percentage of cells of main clone in: Bone marrow	Spleen	Thymus	Lymph node	Total cells examined
549	950r	CBA	Rat	44	−*	54	−	+*	128
1025	850r	CBA	Rat	177	12	16	−	32	335
1433	850r	CBA	Rat	125	+	55	30	−	165
1440	850r	CBA	Rat	127	+	19	−	−	300
1582	800r	C57BL	3HI × T6 mouse	299	95	55	80	67	523

* − = not examined.
\+ = main clone present but not scored quantitatively.

20. In the first experiment animals were sacrificed after 3, 7, 10, 21 and 31 days. Table III shows that in series (a) which received saline only as treatment, the mitotic cells in bone marrow, spleen and thymus were with a few exceptions those of the original donor-type containing the T6 chromosome. The exceptions, 6 cells out of the total 327 counted, were more numerous than in our previous reports [14,15]. Although they are scored as cells of the host, they are still a comparatively unimportant minority of the population and may even represent artefacts, the marker being obscured by other chromosomes. Series (b) and (c) do not differ appreciably up to the 10th day, but series (c) is incomplete because of death of two animals, one from accident and the other for undetermined reasons. At 3 days after the administration of CBA cells, normal or sensitized to the T6 tissue, the cells in bone marrow, spleen and thymus are, like those in series (a), mainly T6 positive. But, at 7 and 10 days, there are definite signs of a change of population in the spleen to T6 negative, that is CBA-type cells. In series (b) there is a similar change to T6 negative cells in bone marrow and thymus at 21 and 31 days.

21. Since the first experiment was incomplete because of the death of the two animals in the group given sensitized spleen, a second similar experiment was carried out. One mouse of series (a), (b) and (c) was sacrificed at 3 days,

two at 3 weeks, two at 5 weeks and one at 14 weeks. These results are also summarized in Table III. The saline control showed T6 positive cells only at all times. In series (b) given normal spleen cells "transpopulation" had been induced by 3 weeks, in one animal in the three tissues, in another only in the spleen. By 6 weeks "transpopulation" was confirmed, though half of the few mitotic cells in the thymus still contained the T6 chromosome. The pattern was quite similar in series (c), given sensitized spleen cells. It is notable that whereas "transpopulation" may have been complete in some animals, in others a component of T6 positive cells persisted.

TABLE III

Ratio of cells containing T6 marker: cells lacking T6 in bone marrow (M), spleen (S) and thymus (T), of CBA/CBA×T6 chimaeras at various times after attempted "induced transpopulation".

	Days after attempted induction	Series (a) given saline			Series (b) given normal CBA spleen			Series (c) given sensitized CBA spleen		
		M	S	T	M	S	T	M	S	T
I	3	45:1	25:0	20:0	41:5	25:0	—	48:0	25:0	19:1
	7	46:1	25:0	20:0	42:3	2:23	19:1	44:1	22:3	20:0
	10	47:1	25:0	19:1	46:0	6:19	19:1	35:7	11:14	17:3
	21	5:2	4:0	—	17:28	0:25	3:17	—	—	—
	31	—	25:0	25:0	—	4:25	1:13	—	—	—
II	3	25:0	25:0	—	25:0	25:0	11:1	25:0	25:0	24:1
	21	25:0	25:0	21:0	22:3	1:24	—	2:23	0:25	—
	22	25:0	25:0	25:0	1:24	3:22	0:19	0:25	2:23	0:3
	42	25:0	25:0	25:0	3:22	1:24	5:5	0:25	0:25	0:1
	43	25:0	25:0	—	0:25	0:25	—	0:25	0:25	—
	98	25:0	25:0	10:0	4:21	3:22	0:1	2:23	4:21	0:17

TABLE IV

Ratio of cells containing T6 marker: cells lacking T6 in spleen (S), thymus (T) and lymph node (L), of CBA/CBA×T6 chimaeras at various times after attempted "induced transpopulation".

	Date after attempted induction	Series (a) given saline			Series (b) given normal CBA lymph node			Series (c) given sensitized CBA lymph node		
		S	T	L	S	T	L	S	T	L
III	2	25:0	—	8:0	25:0	—	3:0	25:0	—	7:0
	21	25:0	—	—	25:0	—	—	25:0	—	15:0
	42	1:0	—	1:0	25:0	—	5:0	25:0	12:8	—
	71	25:0	25:0	15:0	25:0	—	—	20:0	25:0	24:1
	77	25:0	25:0	12:0	25:0	6:0	—	25:0	25:0	6:0

22. In the third experiment, suspensions of lymph node cells were injected instead of spleen cells. "Transpopulation" was not induced by this procedure; only in the thymus of one animal was more than an occasional cell seen which apparently lacked the T6 chromosome. (Table IV).

DISCUSSION

Identification of Clones

23. It is entirely possible, though not proven, that the replacement of the donated cells by those of the host could be a product of a competitive growths in other words, that the cells of the donor's type are less competent in the host's environment than the radiation-damaged cells of the host. If the host's cells simply outgrow the cells of the graft, could then one clone of host cells not outgrow another? Making use of the data of Russell and Major [22] on radiation-induced somatic mutation-rates in the skin of the foetal mouse and of Carter [23] on the probable number of mutable loci in the mouse genome, it is simple to estimate that each cell of the reticuloendothelial system which survives 950 rad of X-irradiation would carry, on the average, about 14 new mutant genes, provided, of course, that all loci are equally mutable and that radiation sensitivity to mutation of the various somatic tissues does not differ appreciably. This might well provide sufficient genetic diversity between clones to account for differential growth. The fact that, in M1582, 95% of the dividing cells of the bone marrow were all of one clone does suggest differential growth. Even if only two ancestral cells contributed to the reappearance of host-type cells, the descendants of the one are vastly more numerous than the descendants of the other. Sampling from a focus of cells all of the same type is ruled out as an alternative explanation, since marrow was taken from both femurs, mixed and handled as a cell-suspension. However, even if only two surviving host cells do contribute to the regeneration of host tissue, it does not follow that their multiplication should commence at the same time; and if, for purposes of argument, the mean interval between successive mitoses is taken to be 24 hrs [24], then it would only require one cell to be 4 days later than the other to account for the observation of 95% cells all of one kind. The problem of whether competition does or does not occur between different clones—other clones will be present, of course, although their presence need not be betrayed by visible chromosome markers—must therefore be left open at present. Nevertheless the additional data reported here support our previous conclusion [15] that relatively few cells contribute to the regeneration of host-tissue.

24. The data of Table II also reinforce the conclusion that cells of host origin are capable of moving about very widely within the body during the process of conversion of the "grafted" myeloid and lymphoid tissue back to tissue of host type. Again referring to the results from mouse M1582, the data reinforce our previous belief in the monophyletic theory of haemopoiesis, namely that all cells of the myeloid and lymphoid series stem from a single progenitor, the haemocytoblast. If this can occur during regeneration of host-type tissue, could it not also occur during the initial colonization of the donated cells? And furthermore in strictly physiological haemopoiesis? A method, by which the value of chromosome rearrangements as markers could be combined

with the preservation of sufficient cytoplasmic detail to permit the identification of the cellular types of the haemotologist might go a long way towards ascertaining the answers.

"Induced Transpopulation" of Homologous Chimaeras

25. These experiments were planned on our hypothesis [15] that the final stable population of cells in the blood-forming tissues of radiation-chimaeras would be those cells most physiologically adapted to the environment; and, so far as they go, the results are not discordant with the thesis. In our previous work [14, 15] it was shown that 3HI×T6 cells were a stable population in the CBA/T6+ chimaera, whereas rat cells were not, so that in the latter case "spontaneous reversion" might occur. This is not due merely to the heterologous matching. As noted in Table II even homologous radiation-chimaeras may spontaneously revert, perhaps since the degree by which homologous cells diverge can vary. In the mouse the most powerful tissue-antigen determining histocompatibility is the H–2. Mice of CBA/H strain are homozygous H–2^k: 3HI mice are somewhat similar since the two parental strains C3H/H and 101/H both contain the K antigen [25]. Whilst the T6 component is genetically heterogeneous, the F_1 (3HI×T6) may have more antigens in common at the H–2 locus with CBA than with C57BL/H(H–2^b). When the latter is host there can be a double dose incompatibility. This may explain why cells of the donor (3HI×T6) are an unstable population in C57BL but stable in CBA mice.

26. In the present experiments substituting (CBA×T6)F_1 mice as the donors to CBA should have made for still greater stability of the donated tissue. Certainly in each control series (a) there was no suggestion of "spontaneous reversion". The few cells in which the T6 chromosome were not found may well, as has been pointed out, be artefactual negatives, but if they are truly cells of the host, having recovered from the irradiation, they show no signs of greater physiological competence than the (CBA×T6) donor cells and are always a small minority.

27. An attempted "transpopulation" of the functioning blood-forming tissues back to CBA should, therefore, require cells physiologically more competent than either irradiated host cells or normal, related but not identical, donor cells. From the experiments involving treatment with CBA spleen-cells, we conclude that CBA spleen-cells have this greater competence. These features are notable:

(i) Whereas the mixed cells of lymph follicles and myeloid pulp in spleen are effective in quite small doses (7 mg/mouse), lymphoid cells alone from lymph nodes in such quantities are not effective. The experiments do not exclude that "transpopulation" might result from larger doses of lymph tissue. The present indication, however, is that it is myeloid cells which are most effective and, from the earlier discussion, perhaps only very few clones are required.

(ii) There is no indication from the results that spleen cells from CBA animals sensitized to the T6 antigens are any more efficacious in hastening the "transpopulation" of the tissues with elimination of the T6

bearing cells than normal CBA spleen cells. This is a further point in favour of the argument that it is not the lymphoid or plasmatoid cells which are the prime movers.

(iii) The "transpopulation" may apparently be complete or only sub-total. In a number of instances a few cells containing the T6 marker persisted. We attach strong weight to this positive evidence of the identity of this cell just as we tend to discount negative evidence when the marker is not seen. This adds to the thesis that the "transpopulation" was effected not by an immune reaction but in virtue of the postulated greater physiological competence.

SUMMARY AND CONCLUSIONS

28. The haemopoietic and lymphopoietic tissues of established homologous and heterologous radiation-chimaeras may revert spontaneously from donor-type to host-type. This reversion may stem from very few cell-clones many of which can be identified cytologically by the presence of characteristic chromosomal rearrangements in mitotic cells.

29. Other homologous radiation-chimaeras may be very stable in that the donor tissue persists and spontaneous reversion does not occur. "Transpopulation" of these haemopoietic and lymphopoietic tissues back to host-type tissue has been effected by the intravenous injection of spleen, but not so far by lymph node, cells isologous with the host.

30. It is concluded that:

(a) both spontaneous reversion and induced "transpopulation" are due to the superior physiological competence of the finally predominating cell-line rather than mastery through a reaction of immunity,

(b) further evidence accumulates of the common ancestors of myeloid and lymphoid cells.

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