Title	Compartments of Leukaemogenic Response to Radiation
Author(s)	Ilbery, P.L.T.
Citation	日本医学放射線学会雑誌. 1971, 31(4), p. 331-339
Version Type	VoR
URL	https://hdl.handle.net/11094/16109
rights	
Note	

Compartments of Leukaemogenic Response to Radiation[1]

P.L.T. Ilbery[2]

放射線による白血病の誘発の諸因子

（昭和46年3月20日受付）

放射線照射したマウスに発生した白血病の染色体像を調べると 100例中88例に異常染色体または染色体数 の 異常が見 られた．照射後骨髄か胎児の肝臓移植を行なうと白血病発生の頻度が減少した．あらかじめ，染色体に特徴をもつたもので移植すると，移植後生じた癌には特殊染色体は見られず，白血病は照射した 細胞から生 じたと言える．これは胸腺摘出と分割照射後に胸腺移植を行なつた時，生じた白血病が移植した照射および非照射胸腺細胞から成り立つていると言う，昔から良く知られた例と全く対照的である．なお，本実験では照射した骨髄細胞を移植した時，それに伴つた胸腺細胞が，胸腺中で増殖して癌となる．ところが非照射骨髄細胞を移植した時には癌を生じないばかりでなく癌の発生を抑制する．

以上は体細胞に起こる突然変異，免疫抑制，コロニー生成および変異による除去の立場から議論された．そして骨髄中の一因子が胸腺の免疫力を回復させることによつて，放射線照射による白血病発生が防止できるのではないかと言う方向にもつていつた．

Abstract

By cytogenetic methods 88 of 100 radiation induced mouse leukaemias were seen to be composed of significant numbers of cells carrying abnormalities of chromosome number and/or form. The incidence of leukaemia following the leukaemogenic schedule of radiation was lowered by grafting bone marrow or foetal liver. When these grafts, suitably labelled chromosomally, were used post radiation to impede the onset of radioleukaemia, neoplasia supervened only in irradiated cells. The finding is in contrast to the incidence from either irradiated or unirradiated cells in thymic grafts after thymectomy and fractionated radiation. Thymic cells in association with irradiated bone marrow cells colonising the thymus became neoplastic but non-irradiated bone marrow cells colonising an irradiated thymus were not rendered neoplastic and indeed had an inhibitory effect in the incidence of leukaemia. The findings are discussed in terms of somatic mutation, immunological suppression and clonal explosion, and transformation elimination; and orientated to prevention of radiation induced leukaemia through a bone marrow factor in restoration of thymic competence.

Introduction

C57BL mice have a high incidence of radiation induced leukaemia with a relatively low incidence of spontaneous leukaemia.　Wallis et al. (1966) showed an incidence of about 70 per cent in the (C57BL × CBA) F_1 hybrid.　Ilbery and Barnes (1969) using 4 fractions of 200 rad ^{60}Co gamma radiation at intervals of 4 days achieved an incidence of 67 per cent in (C57BL × CBA. T6T6) F_1 and (C57BL × CBA/H) F_1 hybrids.　Female mice were used because of the greater susceptibility of their sex to radiation induced leukaemia.　The leukaemia induced in this hybrid makes its appearance in the thymus.　Animals sacrificed in extremis with respiratory distress sometimes only show macroscopic involvement of the thymus. Nevertheless it is usual at this stage to obtain passage of the leukaemia by using either blood, lymph node, bone marrow, liver or spleen as well as thymoma.

Thymectomy abolishes the leukaemic response to radiation.　Subcutaneous implantation of a thymic graft restores leukaemia induction in the thymectomized mouse exposed to a leukaemogenic schedule of radiation (Law and Potter, 1956; Kaplan et al., 1956).　In this thymectomized irradiated thymic grafted mouse leukaemia model it has been shown that when host and donor are distinguished by means of a chromosome label that in about half the resulting leukaemias the malignant cell can be demonstrated to have arisen from the thymic graft (Ilbery, 1958; Barnes et al., 1959).　The thymic graft has not been exposed directly to the effects of radiation and this fact is used as a keystone for an indirect viral causation hypothesis in leukaemogenesis.

A series of 100 radiation induced mouse leukaemias have been examined cytogenetically.　The influence of somatic mutation, immunological suppression and clonal explosion, and elimination of cells capable of neoplastic transformation is discussed in the light of the following results.

Materials and Methods

Gamma rays from radiocobalt were the source of radiation.　The dose rate from the Royal Prince Alfred Hospital Theratron Unit varied from 48 to 83 rads/min. at 75 cm source/target distance.

The cytogenetic technique employed was a modification of the hypotonic, fixative, air-drying sequence making use of different stains but lately using lacto-acetic-orcein (Barnes and Ilbery, 1968).

Female mice either (C57BL × CBA/H) F_1 or (C57BL × CBA.T6T6) F_1 were subjected to radiation at the age of 30 to 40 days.　CBA/H and CBA.T6T6 are syngeneic and skin grafts have been made down the breeding lines with compatible results.　Thus the hybrids (C57BL × CBA /H)F_1 and (C57BL × CBA. T6T6) F_1 can also be regarded as syngeneic.

Results and Discussion

Somatic mutation

88 out of the first one hundred radiation induced mouse leukaemias, examined cytogenetically in this laboratory, have revealed abnormal classes of chromosome sets as defined.　The laboratory has considered an hyperdiploid class to be present if more than 5 per cent of the total cells examined belong to it, and the presence of an hypodiploid class is inferred by the presence of 10 per cent of cells carrying the abnormal number.　The findings shown graphically in Figure 1 probably represent merely the visible structurally altered moiety of massive genetic variation under these experimental conditions.　The figure does not allow for superimposition of the further variation seen as structural alteration of the chromosomes (examples
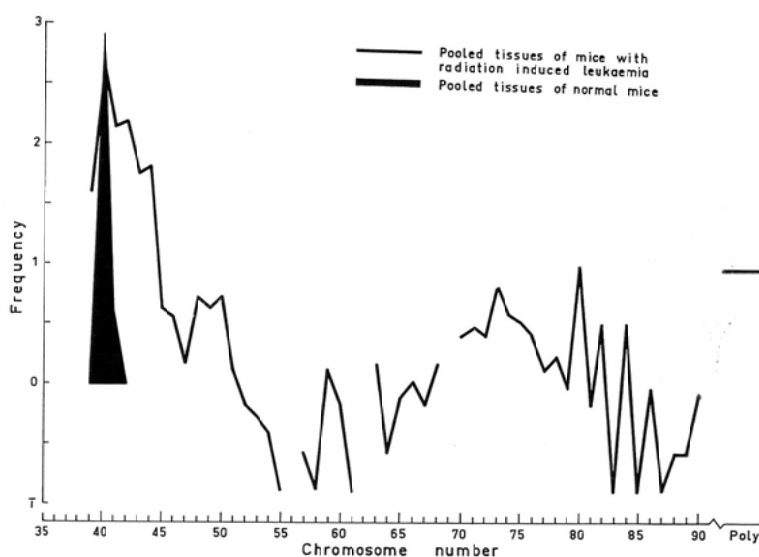
Fig. 1. Log. frequency per thousand cells of chromosome counts of various classes from 100 cases of radiation induced mouse leukaemia (compared with the distribution of classes within normal tissues).
—— pooled tissues of mice with raidation induced leukaemia
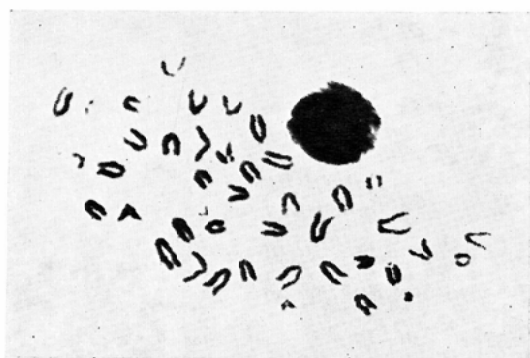▬ pooled tissues of normal mice.

Fig. 2. Complement of 42 chromosomes in a cell from a thymoma induced following the leukaemogenic radiation schedule. Three abnormally small chromosomes are present in addition to the T6 marker seen near the centre of the spread.

Fig. 3. Complement of 43 chromosomes from another thymoma cell. A heterchromatic marker chromosome is seen towards the periphery of the spread at the left and an abnormally large chromosome towards the lower right.

of such aberrations of form are given in Figures 2 and 3). Not only does the leukaemia seem to arise macroscopically within the thymus but there is a more consistent variation from the normal mode within the cells of the thymus than in the other lymphoid and haemopoietic organs. The usual range of the variation within thymoma material is near diploid (39–44). When the results of the cytogenetic findings of all the

tissues from the propositi of radiation induced leukaemia are lumped together the near diploid distribution is seen to be spread and a lesser peak of variability is observed in the hypo-tetraploid range (70–80). Whenever distinctive classes or clones were seen in any of the tissues routinely examined (thymus, lymph node, bone marrow, spleen or blood), the thymus was abnormal in containing such aberrations. Inspection of the aberrations of chromosome number and form within the thymus and the distribution of distinctive clones and classes within the other tissues are consistent with an origin of the radiation induced neoplasms within the thymus (Ilbery et al., 1968).

Even in the preleukaemic phase of the disease there are observable variations in chromosome number and form (Ilbery et al., 1963; Joneja and Stich, 1965). The former serially sacrificed mice exposed to the leukaemogenic schedule of radiation and classified those mice as preleukaemic in which the thymic weight was less than 70 mg and when transplantation of half the thymus gland did not yield passage leukaemia. Of 19 mice satisfying these criteria cytogenetic examination of the other half of the thymus revealed 9 with an increased chromosome number either as the mode or as the presence of significant classes as defined while 4 of these thymic samples contained chromosome complements with a mode of greater than 40 chromosomes. A further 3 thymic samples showed the presence of clones with apparently unbalanced structural anomalies.

Immunological suppression and clonal explosion.

It is possible in R mice, in terms of their ability to withstand challenge with Sarcoma 1, to titrate their immunological suppression to graded doses of whole body radiation (Ilbery, Koller and Loutit, 1958). When challenged one week after 250 w.b.r., 90 per cent of the mice succumbed and even after 30 days 20 per cent failed to withstand the challenge of foreign tumour cells. In the immunological hiatus following 4 fractions of 200 rads w.b.r. it is readily understandable that a mutant cell could outgrow the immunological capability of the host.

The incidence of radiation induced leukaemia has been lowered by post radiation treatment with bone marrow (Kaplan et al., 1953) and spleen (Lorenz et al., 1953). Using the syngeneic mice hybrids (C57BL × CBA/H) F_1 and (C57BL × CBA.T6T6) F_1, in which no proliferative advantage should be present in transplantation experiments, Ilbery (1965) found the appearance of dividing cells having the chromosome characteristic of the donor almost exclusively, following administration of foetal liver cells to mice receiving a leukaemogenic schedule of radiation, in the first month post treatment. The ensuing lowered incidence of radiation induced leukaemia could be attributed simply to displacement of the radiation damaged and potentially neoplastic cells of the host by unirradiated graft cells. However the pattern of repopulation suggested otherwise. The order of appearance of the dividing cells within the hosts' tissues showed there was a delay of some three to four weeks before a rising mitotic rate was seen in the thymus. The situation was not changed by supplementing, at the time of the foetal liver administration, with immature thymocytes free or as whole organ graft, nor with adult lymphoid material. Gowans and Knight (1964) concluded that failure of intravenously given lymphoid cells to enter the thymus was a function of the homing of these cells into lymph nodes because of the special affinity of small lymphocytes for the endothelium of the postcapillary venules. It was postulated that it was a cell derived from the recolonized haemopoietic stores that gains entry to the thymus rather than a thymocyte introduced at the

time of chimaerism by simple population pressures. The cells of the regenerated thymus appear histologically to be typical thymocytes. This being so, it appears that stem cells from foetal liver (or bone marrow) mature in the lymphoid direction on entry into, and presumably under the control of the thymus. The mitotic rate in lymph nodes remained low throughout the experiment. Increase in lymph node weight was concurrent with decrease in mitosis in the thymus. However the mitotic rate in the bone marrow was consistently high so that the inference of the origin of the lymphocytes from thymus with distribution centrifugally to lymph nodes could not be linked as in the findings of Good et al. (1962) and Porter and Cooper (1962). Further Harris and Ford (1964) have shown conclusively that cells derived from a primary host that had sojourned in a thymic graft had acquired the capacity to migrate to the lymph nodes of a new host.

Along with the delay in the repair of the thymus there is considerable delay in the restoration of the peripheral blood count. In this laboratory it has been clearly seen that following resuscitation of the lethally irradiated mouse with haemopoietic material, one month elapses before the white count has assumed normal levels. However it is usually two months or more before it resumes the normal proportion of the lymphocyte/neutrophil ratio and this is true whether there has been supplementation with adult or new born thymocytes or lymphocytes.

The immunological impairment in this period, it is assumed, enables classes or clones of cells normally subject to control by the host to multiply to levels where they can be recognized in the preleukaemic stage by the rather gross method of cytogenetic examination. Yet as has been shown, these cellular enclaves are not capable at this stage of initiating growth on passage to intact, susceptible mice. The explanation for the varying period over which induction of this radiation induced leukaemia spreads may be that although such enclaves are tolerated because of disruption of the hosts' immune mechanisms, the time to switch over to neoplastic clonal explosion of their inherent potential is as varied as their cytogenetic appearance. Nevertheless within the induction period there are two well defined peaks, one at about 200 days and a lesser one at about 300 days post radiation. This unexplained pattern remains regardless of whether leukaemia follows post radiation treatment with haemopoietic or lymphoid material.

Post radiation treatment with lymphoid material appears to be without effect on the incidence of radiation induced leukaemia. Even whole lymph node graft made beneath the kidney capsule was ineffective. On the contrary preservation of a small amount of lymphoid material by shielding of the lower abdominal wall node during radiation was effective (Ilbery, 1967).

Transformation elimination

A question in leukaemogenesis that needs an answer is the reason for the failure to devise schedules of radiation uniformly leukaemogenic to all mice at risk. Kaplan and Brown (1952) despite much experimentation with dose and dose fractionation were not able to achieve total leukaemia induction. In this laboratory the final fraction of the schedule of 200 rad on each of 4 occasions at 4 day intervals was increased in 100 rad steps to assess dose-response relationships. It was found with increasingly larger final increments of whole body radiation the incidence of leukaemia diminished (Table 1). Figure 4 shows the increasing incidence of death from aplastic anaemia with loading of the final increment and the relationship between a decreasing incidence of leukaemia and increasing numbers of deaths from aplastic anaemia.

Table 1.　Mortality Following Leukaemogenic Schedule of Radiation with Increasing
Final Increment

Final radiation increment (rad)	No. of mice	Dead from leukaemia	Dead from aplastic anaemia
200	100	66	7
300	6	4	–
400	10	4	1
500	6	2	–
600	5	2	–
700	5	1	1
800	5	–	–
900	9	–	·
1000	7	–	7
1100	5	–	5

Mole (1958) had drawn attention to the inhibition in development of leukaemia if whole body radiation of mice was followed by a single large though sub-lethal dose of whole body radiation and the effect is visible in these results at about 800 rad.　Ten mice received a final fraction of 1000 rad and were then resuscitated with an intravenous injection of foetal liver.　Four out of ten surviving aplastic anaemia and living for a year did not develop leukaemia.

It seems clear in this final compartment in the leukaemogenic response to radiation that there is a diminishing return in leukaemia induction as the population of cells capable of transformation to neoplasia falls.

Of course thymectomy by eliminating the cells at risk abolishes the leukaemic response to radiation. Ito et al. (1969) reported that induction of leukaemia by ^{90}Sr was not inhibited by thymectomy.　They postulated that X-irradiation produces progenitors of leukaemic cells in bone marrow and these cells migrate into the injured thymus where they proliferate, while the similar progenitor cells can be produced in bone marrow by direct effects of ^{90}Sr.

Comment on compartmental response

It has been suggested that somatic mutation was of primary importance in the onset of neoplasia(Boveri, 1914).　More probably there is an association for such changes with tumour progression (Hauschka, 1961).　Nevertheless in the cytogenetic studies of the thymus in radiation induced leukaemia and in its preleukaemic phase as outlined here, it seems that observable variations in chromosome number and form accompany an early stage of leukaemia induction.　Are these changes sufficient in themselves to initiate leukaemia induction?　If neoplastic transformation is essentially at gene level then these relatively gross cytogenetic changes must reflect a neoplastic potential.　The first compartment shows therefore a linear response at a low probability level to increasing radiation exposure.　Once the immune mechanisms fails as indicated schematically in Figure 5, there is a release of variant cells from restraint.　Clonal explosion (Fig. 2 and 3) under these circumstances is to be expected of cells with greater growth potentials and is an observed fact seen through the eyes of the cytogeneticist classifying the flood of near diploid cells containing distinctive clones and classes in leukaemogenesis.
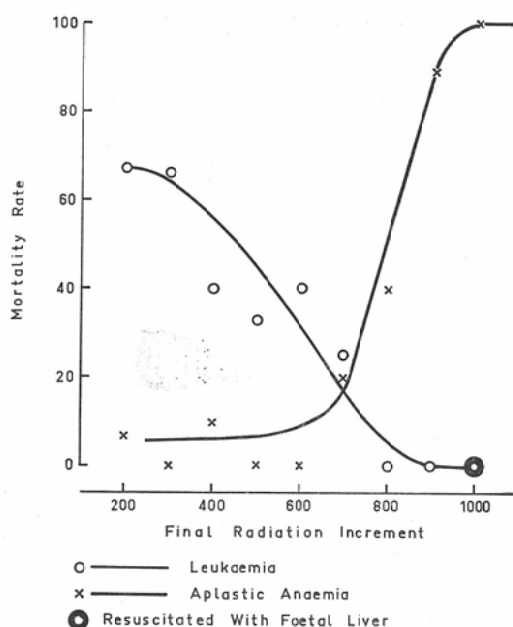
Fig. 4. Relationship of aplastic anaemia to radiation induced leukaemia following an increase in the final radiation increment of 4 × 200 rad w.b.r.

Trends in Leukaemia Induction by Compartments of Response

------ Somatic Mutation
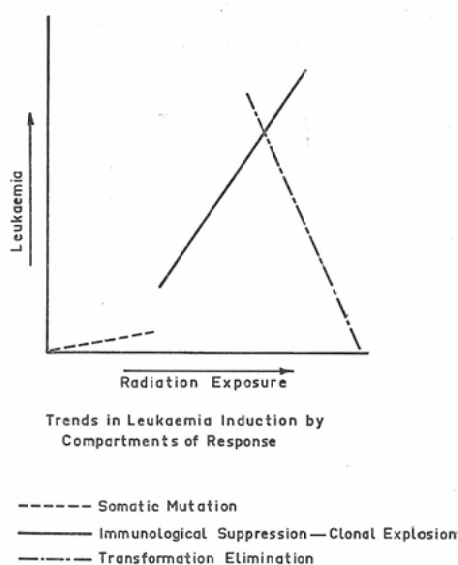——— Immunological Suppression—Clonal Explosion
—·—·— Transformation Elimination

Fig. 5. Trends in leukaemia induction by compartments of response

Viral activation. Activation of latent virus might well determine the previously indicated unexplained peaking of leukaemia induction at about 200 days post radiation. Some features of such a virus have been described by Hoffman and Darveniza (1961). There is support for virus being an initiating agent, for Lieberman and Kaplan (1959) were able to produce leukaemia with extracts from thymus prepared only 68 days after irradiation. Ilbery and Winn (1964) obtained an incidence of 22 per cent generalized leukaemia in (C57BL × T6T6) F_1 hybrids inoculated neonatally with cell free extracts of C57BL radiation-induced leukaemias. The chromosome marker denied origin form cellular transfer. Chromosome aberrations of number and form of the type seen in radiogenic leukaemia were also observed in the leukaemas which followed inoculation with cell-free extracts. The mean time to development of this leukaemia (600 days) suggests that a lower concentration of the leukaemogenic agent can be obtained by preparing cell-free extracts from leukaemic tissues than that which is present following whole body radiation.

The presence of virus following whole body radiation is an explanation for the origin of the neoplastic cell in some cases from host type cells and in other cases from the cells of the thymic graft in the thymectomized, irradiated, thymic grafted mouse leukaemia model. Otherwise an explanation has to be advanced for leukaemia induction in the graft cells which had not been exposed to radiation. Miller (1961) using the same technique as Barnes et al. (1959) but replacing radiation with inoculation of leukaemic filtrate passage A of Gross, showed that in four out of five thymic graft neoplasms the cells of origin were of host

type.　Since these mice were inoculated at birth with the sub-cellular agent, the leukaemogenic influence has been present in the host cells longer than in the radiation experiment where the latent virus, it is argued, is not activated until 30–40 days after birth at the time the first fraction of radiation is commenced. Since the thymus is grafted immediately following the last fraction of radiation there is an almost equal temporal influence on host and graft type cells commensurate with the incidence of host and graft type tumours.

How does the virus achieve neoplastic response among the massive genetic variation (of which the cytogenetic aberrations represent the portion of the iceberg merely above water) within the thymus? Does it increase the variation until the neoplastic response is elicited, is it the essential factor in leukaemogenesis (some would have that leukaemogenesis and viral induction are synonomous), or does it act by further lowering the immune response (perhaps by blocking immunological processes)?

Bone marrow factor.　The origin of neoplastic cells following prophylaxis of radiation induced mouse leukaemia with haemopoietic material is invariably from the irradiated tissues of the host (Ilbery and Barnes, 1969).　What is the explanation for neoplastic chromosomally altered cells of host and donor origin in the thymectomized, irradiated, thymic grafted situation and of host only in the irradiated, bone marrow grafted situation?　A simple explanation would be to assume the presence in bone marrow of some factor able to suppress the proliferation of altered cells multiplying within the thymic environment. The factor might be subcellular or deficiency of a particular bone marrow cell.　It is clear from our experiments that colonization of the thymus is effected by a cell derived from the bone marrow.　Since there is a delay of some weeks before bone marrow grafted cells colonise and actively divide within the thymus, in the case of the irradiated bone marrow grafted mice abnormal clones of thymic cells may have already arisen.　In the case of the thymic grafted mice the irradiated bone marrow cells, deficient in the factor following radiation exposure, not only fail to suppress the development of abnormal thymus derived clones but also alter themselves without restraint in the thymic environment.

Orientation towards a bone marrow factor rationalizes the comparison of the findings in the thymectomized, irradiated, thymic grafted model and the intact, irradiated, bone marrow grafted mouse leukaemia model.　Irradiated bone marrow cells colonizing the thymus and the unirradiated thymus cells themselves become neoplastic but non-irradiated bone marrow cells colonising an irradiated thymus do not undergo neoplastic transformation and indeed exert an inhibitory effect on leukaemia induction.

It appears that prevention of radiation induced leukaemia depends on the availability of a cell derived from bone marrow in restoration of thymic competence.

References

1) Barnes, C.A. and Ilbery, P.L.T.:　Cytogenetic techniques in radiobiological procedures. Aust. Radiol., 12, 395.

2) Barnes, D.W.H., Ford, C.E., Ilbery, P.L.T., Jones. K.W. and Loutit, J.F.:　Murine leukaemia. Acta Un. int. Cancr., 15: 544, 1959.

3) Boveri, T.:　Zur Frage der Entstehung maligner Tumoren. Jena, Fischer, 1914.

4) Good, R.A., Dalmasso, A.P., Martinez, C., Archer, O.K. and Papermaster, B.W.:　The role of the thymus in development of immunologic capacity in rabbits and mice. J. exp. Med., 116: 773, 1962.

5) Gowans, J.L. and Knight, E.J.: The route of recirculation of lymphocytes in the rat. Proc. roy. Soc. B., 159: 257, 1964.

6) Gross, L.: Oncogenic Viruses, Pergamon Press, New York.

7) Harris, J.E. and Ford, C.E.: Cellular traffic of the thymus: experiments with chromosome markers. Nature, 201: 884, 1964.

8) Hauschka, T.S.: The chromosomes in ontogeny and oncogeny. Cancer Res., 21: 957, 1961.

9) Hoffman, H. and Darveniza, N.: Electron microscopic studies of a transplantable, radiation-induced mouse leukaemia. Radiobiology (Ed. P.L.T. Ilbery), Butterworths, London, pp. 161.

10) Ilbery, P.L.T.: Evidence for a direct mechanism in leukaemogenesis. Aust. Atomic Energy Symp., (1958), pp. 681.

11) Ilbery, P.L.T.: Thymic, lymphoid and marrow inocula in cytogenetic repopulation studies of irradiated mice. Aust. J. exp. Biol. med. Sci., 43: 579, 1965.

12) Ilbery, P.L.T.: Prevention of radioleukaemia by lymph node shielding. Nature, 215: 655, 1967.

13) Ilbery, P.L.T. and Winn, S.M.: Indirect transfer of radiogenic lymphoma. Aust. J. exp. Biol. Med. Sci., 42: 133, 1964.

14) Ilbery, P.L.T. and Barnes, C.A.: Origin of neoplastic cells following prophylaxis of radiation induced mouse leukaemia. Int. J. Cancer (in press).

15) Ilbery, P.L.T., Moore, P.A., Winn, S.M. and Ford, C.E.: Chromosomes in murine pre-leukaemia. In Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation, Ed. by R.J.C. Harris. Academic. London, (1963), pp. 83.

16) Ilbery, P.L.T., Winn, S.M. and Barnes, C.A.: Cytogenetic variability in radiation induced mouse leukaemia. Brit. J. Cancer, 22: 743, 1968.

17) Ito, T., Yokoro, K., Ito, A. and Nishihara, E.: A comparative study of the leukaemogenic effects of strontium-90 and X-rays in mice. Proc. Soc. exp. Biol. (N.Y.), 130: 345.

18) Joneja, M.G. and Stich, H.F.: Chromosomes of tumour cells. IV. Cell population changes in thymus, spleen and bone marrow during X-ray induced leukemogensis in C57BL-6J mice. J. nat. Cancer Inst., 35: 421, 1965.

19) Kaplan, H.S. and Brown, M.B.: A quantitative dose-response study of lymphoid-tumour development in irradiated C57 black mice. J. nat. Cancer Inst., 13: 185, 1952.

20) Kaplan, H.S., Brown, M.B. and Paull, J.: Influence of bone marrow injections on involution and neoplasia of mouse thymus after systemic irradiation. J. nat. Cancer Inst., 14: 303, 1953.

21) Kaplan, H.S., Carnes, W.H., Brown, M. and Hirsch, B.: Tumour incidence and morphology in mice bearing non-irradiated thymic grafts. Cancer Res., 16: 422, 1956.

22) Law, L.W. and Potter, M.: Behaviour in transplants of thymocytic neoplasms arising from parental thymic graft in irradiated thymectomized hybrid mice. Proc. nat. Acad. Sci. (Wash.), 42: 160, 1956.

23) Lieberman, M. and Kaplan, H.S.: Leukaemogenic activity of filtrates from radiation-induced lymphoid tumors of mice. Science, 130: 387, 1959.

24) Lorenz, Egon, Congdon, C.C. and Uphoff, D.E.: Prevention of irradiation-induced lymphoid tumours in C57BL mice. J. nat. Cancer Inst., 14: 291, 1953.

25) Miller, J.F.A.P.: Analysis the thymus influence in leukaemogenesis. Nature, 191: 248, 1961.

26) Mole, R.H.: The development of leukaemia in irradiated animals. Brit. Med. Bull., 14: 174, 1958.

27) Porter, K.A. and Cooper, E.: Transformation of adult allogeneic small lymphocytes after transfusion into newborn rats. J. exp. Med., 115: 997, 1962.

28) Schwartz, R.A. and Beldotti, L.: Malignant lymphomas following allogeneic disease: transition from an immunological to a neoplastic disorder. Science, 149: 1511, 1965.

29) Wallis, V., Davies, A.J.S. and Koller, P.C.: Inhibition of radiation induced leukaemia by the injection of haematopoietic tissue: a study of chimaerism. Nature, 210: 500, 1966.