Genet. Res., Camb. (1983), **42**, *pp.* 29–39
With 1 plate and 3 text-figures
Printed in Great Britain

Haematopoietic role for Patch (*Ph*) revealed by new *W* mutant (W^{ct}) in mice

By J. F. LOUTIT† and B. M. CATTANACH

M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon, OX11 0RD

(*Received 20 December 1982 and in revised form 14 March 1983*)

SUMMARY

A new mutant (W^{ct}) has been identified at the *W* locus of the mouse. The homozygote is poorly viable. Whereas the heterozygote ($W^{ct}/+$) is only mildly anaemic like $W^{v}/+$, the double heterozygote $W^{ct}+/+Ph$ is considerably more anaemic than $W^{v}+/+Ph$ and it and $W^{sh}+/+Ph$ have significantly raised leucocyte counts. $W^{ct}+/+Ph$ is also unduly radiosensitive to whole body X-irradiation, 50% dying from haematopoietic failure at a dose of $4{\cdot}59 \pm 0{\cdot}14$ Gy,* whereas the median for $W^{ct}/+$ was $6{\cdot}49 \pm 0{\cdot}28$ Gy. Serial blood counts of mice after low- or sub-lethal doses of X-rays revealed significantly more profound depression of counts of both red cells and leucocytes in $W^{ct}/+$, and more notably in $W^{ct}+/+Ph$, than in $+/+$ or $W^{sh}/+$ (haematologically normal) iso-dosed mice. We conclude that control of haematopoiesis by chromosome 5 is not confined to the *W* locus but is shared by the linked gene *Ph* (and perhaps *Rw*) and that expression of the change is not limited to the erythron but involves the pluripotent haematopoietic stem cell.

1. INTRODUCTION

Patch (*Ph*) was reported as a dominant spotting gene linked with *W* on chromosome 5, by Grüneberg & Truslove (1960). They suspected it might have a role like *W* in haematopoiesis but the only positive evidence was a modest increase in the mild macrocytic anaemia of $W^{v}/+$ when doubly heterozygous with *Ph*.

We show that *Ph* as a double heterozygote with a new mutant W^{ct} causes marked anaemia, modest leucocytosis and undue sensitivity to death from haematopoietic failure after whole body X-irradiation.

2. MATERIALS AND METHODS

(i) *Mice*

Cattanach's dominant spotting W^{ct}. This spontaneous mutant was first detected in a single male from a closed breeding stock in which hypogonadal, *hpg* (Cattanach

* Abbreviations used: Gy, Gray, S.I. unit of absorbed dose of radiation, = 100 rad; RBC, red blood cells; WBC, white blood cells; PCV, packed cell volume of red blood cells; MCV, mean corpuscular volume; CFUs, colony forming units (spleen).

† Visitor with grant from Medical Research Council.

et al. 1977) was being maintained (Cattanach, 1982). The genetic background was a mixture of C3H/HeH and 101/H, the stock earlier having been maintained for several generations by crossing to F_1 (C3H/HeH × 101/H) hybrid mice. The phenotype of this individual and of $W^{ct}/+$ descendants was that of a heterozygote for a dominant spotting (W) allele, the white spotting being only a little more extensive than that of viable dominant spotting (W^v) heterozygotes, on similar backgrounds. The line was maintained in crosses to hybrid (C3H/HeH × 101/H)F_1 mice.

Extreme dominant spotting, W^e (Cattanach, 1978), viable dominant spotting W^v (Little & Cloudman, 1937), sash, W^{sh} (Lyon & Glenister, 1982), patch *Ph* (Grüneberg & Truslove, 1960) and rump-white, *Rw* (Searle, 1965) were also used in the experiments. These mutants were similarly maintained in stocks of a mixed C3H/HeH–101/H genetic background. Wild type mice were the C3H/HeH × 101/H F_1 hybrids.

(ii) *Haematological methods*

Blood. This was obtained by phlebotomy of the tail.

Counting. Total counts of red corpuscles (RBC) and leucocytes (WBC) were performed by standard methods involving dilution of well mixed blood and counting by eye in a haemocytometer. Differential white cell counts were also made together with total counts in the haemocytometer. Provided abnormal cells are not a feature, the nucleated cells can be classified into 3 groups (*a*) polymorphonuclear granulocytes, with refractile nuclear membrane; (*b*) mononuclear lymphocytes mostly small, occasionally large, with round nuclei, usually refractile nucleolus, and scanty cytoplasm; (*c*) mononuclear monocytes with bilobed nucleus, each half having a dark central spot, and more abundant cytoplasm than all except large lymphocytes. This method of differentiation of cells in suspension avoids the disadvantages of the standard method of differentiation in stained films, namely uneven spreading, ruptured 'smear' cells and, in case of gross leucopenia, rarity of countable cells. Minimal counts of 400 red corpuscles and 100 white cells (except in extreme leucopenia) were made and gave statistical 'errors' of 5 and 10% respectively for these totals.

Packed cell volumes. PCV were determined in heparinized capillaries with a Hawksley microhaematocrit.

Statistical analyses. These were performed by our professional statistician, Mr D. Papworth. Means and their standard errors were calculated by standard methods but the numerical comparisons were made with non-parametric tests, the Mann-Whitney for two sample comparisons, the Kruskal-Wallis for more than two comparisons. Median lethal doses of X-irradiation were estimated from probit regression lines fitted by the method of maximum-likelihood (Finney, 1952).

(iii) *X-irradiation*

Mice were totally X-irradiated by a standard method of the laboratory (Brown, Corp & Westgarth, 1960) the factors being 250 kV, 14 mA, HVL 1·1 mm Cu, dose rate $\sim 0{\cdot}5$ Gy min^{-1}.

3. RESULTS

W^{ct} *inheritance.* Crosses of the original mutant $W^{ct}/+$ male with $+/+$ females produced 41 offspring of which 20 were $+/+$ and 21 resembled the father (Table 1(1)). Both sexes were equally represented. Intercrosses of the $W^{ct}/+$ progeny produced 38 anaemic black eyed white W^{ct}/W^{ct} (classified at birth), 129 $W^{ct}/+$ and 57 $+/+$ (classified at weaning) (Table 1(2)). The frequencies of heterozygotes and wild types accord well with the 2:1 ratio expected ($\chi^2_1 = 0{\cdot}64$, $P \sim 0{\cdot}4$);

Table 1. *Segregation of* W^{ct} *in various types of crosses*

Mating	Classified offspring				
	W^{ct}/W^{ct}	$+/+$	$W^{ct}/+$	$W^{ct}+/+Rw$	$++/++Rw$
(1) $W^{ct}/+ \times +/+$	—	20	21	—	—
(2) $W^{ct}/+ \times W^{ct}/+$	38	57	129	—	—
(3) $W^{ct}/++ \times ++/Rw$	—	16	17	12	14
(4) $W^{ct}+/+Rw \times ++/++$	—	—	101	—	83
	Compound			Double heterozygote	
	W^{ct}/W^{*}	$W^{ct}/+$	$W^{*}/+$	$Ph/+$	$W^{ct}+/+Ph$ $W^{*}+/+/Ph$
(5) $W^{ct}/+ \times W^{sh}/W^{sh}$	24	—	30	—	— —
(6) $W^{e}+/+Ph \times W^{ct}+/++$	11	—	11	11	12 —
(7) $W^{v}+/+Ph \times W^{ct}+/++$	10	—	6	15	7 —
(8) $W^{ct}/W^{sh} \times +/+$	—	22	20	—	— —
(9) $W^{ct}+/+Ph \times ++/++$	—	27	—	22	— —
(10) $++/+Ph \times W^{ct}+/W^{sh}+$	—	32	36	—	36 44
(11) $++/+Ph \times W^{ct}+/+Ph$	—	27	—	26	10 —

however, there was clearly a marked shortage of homozygotes. This suggests a reduced pre- or perinatal viability which may be estimated from these data to be about 60% of normal. W^{ct}/W^{ct} survival after birth was also limited, median day of death = 5; most died within 2 weeks, but one survived for 32 days. There was no visible skin or hair pigmentation.

W^{ct}-Rw *combination, linkage.* Crosses of $W^{ct}+/++$ with $++/+$ *Rw* produced 12 near-white, double mutant ($W^{ct}+/+Rw$) progeny with limited pigmentation of ears and some pigmented hairs on dorsal parts of body and in the region of the genitalia, 14 $++/+Rw$, 17 $W^{ct}+/++$ and 16 $++/++$ (Table 1(3)). The viability of double mutants was near normal to weaning with no losses thereafter. Crosses of the double mutant $W^{ct}+/+Rw$ with (C3H × 101)F$_1$ mice have so far produced 101 $W^{ct}+/++$ and 83 $++/+Rw$ progeny (Table 1(4)).

Additional breedings for stock and progeny are also listed in Table 1(5)–(11). From these one may note that:

(*a*) compounds of W^{ct} with other W^{*} mutants, W^{sh}, W^{e} and W^{v} appeared at birth as black-eyed-whites in the expected proportions – 0·5, 0·25 and 0·25 of totals (Table 1, lines 5–7).

(*b*) W^{ct}/W^{sh} was fully viable to maturity and fertile (Table 1(8) and (10)) with the classified progeny approximately in the expected proportion when mated with $+/+$ ~0·5 and with $Ph/+$ ~ 0·25.

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(*c*) W^{ct}/W^{e} and W^{ct}/W^{v} on the other hand were less viable; only 3/11 W^{ct}/W^{e} (6) survived to maturity, 8/10 W^{ct}/W^{v} (7). Four of the latter were tested for fertility (data not shown in Table 1). Each of two males was fertile, producing litters of 4–11 from three females: one then died (thymic lymphosarcoma), the other was killed for operational reasons. The two others were females and each produced a small litter. Both, however, died shortly after a successful lactation.

(*d*) The double heterozygotes $W^{ct}+/+Ph$, inferred as such from matings (6) and (7) of Table 1, resembled $W^{v}+/+Ph$ in distribution of diluted pigment, but this was less extensive, in muzzle and ears with perhaps a few spots elsewhere (see Plate 1). These double heterozygotes, though anaemic (*vide infra*) were fully fertile,

Table 2. *Mean values (±standard error) for peripheral blood red cells of mice of various genotypes*

Genotype	No. of mice	RBC $\times 10^{-9}$ ml	PCV %	MCV μm^3
(1) +/+	11	9·97 (±0·18)	47·45 (±0·56)	47·64 (±0·78)
(2) $W^{ct}/+$	19	8·67 (±0·26)	45·61 (±0·91)	53·2 (±1·2)
(3) W^{ct}/W^{sh}	6	8·28 (±0·22)	44·83 (±0·6)	54·7 (±1·1)
(4) $W^{ct}+/+Ph$	26	6·59 (±0·30)	38·4 (±1·5)	59·0 (±1·1)
$W^{v}/+$	13	8·65 (±0·25)	45·73 (±0·77)	53·1 (±1·0)
$W^{v}+/+Ph$	13	8·03 (±0·35)	43·12 (±0·86)	54·3 (±1·6)
P values				
2 *v.* 1		< 0·002	= 0·10	< 0·002
2 *v.* 3		> 0·10	> 0·10	> 0·10
2 *v.* 4		= 0·000029	= 0·00039	= 0·00084

Table 1 (9) and (11). Their genetic constitution was confirmed in crosses to wild type mice giving 27 $W^{ct}/+$ and 22 $Ph/+$ offspring. Mating (11) with a $Ph/+$ female was anomalous to the extent that it produced only about one half of the expected 20 or so $W^{ct}+/+Ph$ offpsing (the *Ph* homozygote is lethal).

Blood counts. Red cell counts were made on mice aged 2 months or more. The results are listed in Table 2. Compared with +/+ controls $W^{ct}/+$ mice are mildly anaemic ($P < 0{\cdot}002$) with slight macrocytosis ($P < 0{\cdot}002$), but normal PCV ($P > 0{\cdot}10$). The values for individuals were in the ranges $5{\cdot}5–10{\cdot}4 \times 10^{9}$ ml^{-1} for RBC, 35–52 % for PCV and 64–50 μm^3 for MCV (mean corpuscular volume). Six W^{ct}/W^{sh} varied but little about the mean RBC $8{\cdot}3 \times 10^{9}$ ml^{-1}, PCV 45 %, MCV 55 μm^3 and did not differ significantly in any of the means from $W^{ct}/+$ ($P > 0{\cdot}10$).

$W^{ct}+/+Ph$ showed considerable variability about the means – $6{\cdot}6 \times 10^{9}$ ml^{-1} for RBC, 38 % for PCV and 59 μm^3 for MCV with ranges $3{\cdot}8–8{\cdot}8 \times 10^{9}$ ml^{-1}, 22–50 % and 75–49 μm^3 respectively. All measured means were significantly lower than in $W^{ct}/+$ (*P* RBC = 0·000029, PCV = 0·00039, MCV = 0·00084). Counts for $W^{v}/+$ and $W^{v}+/+Ph$ are given for comparison.

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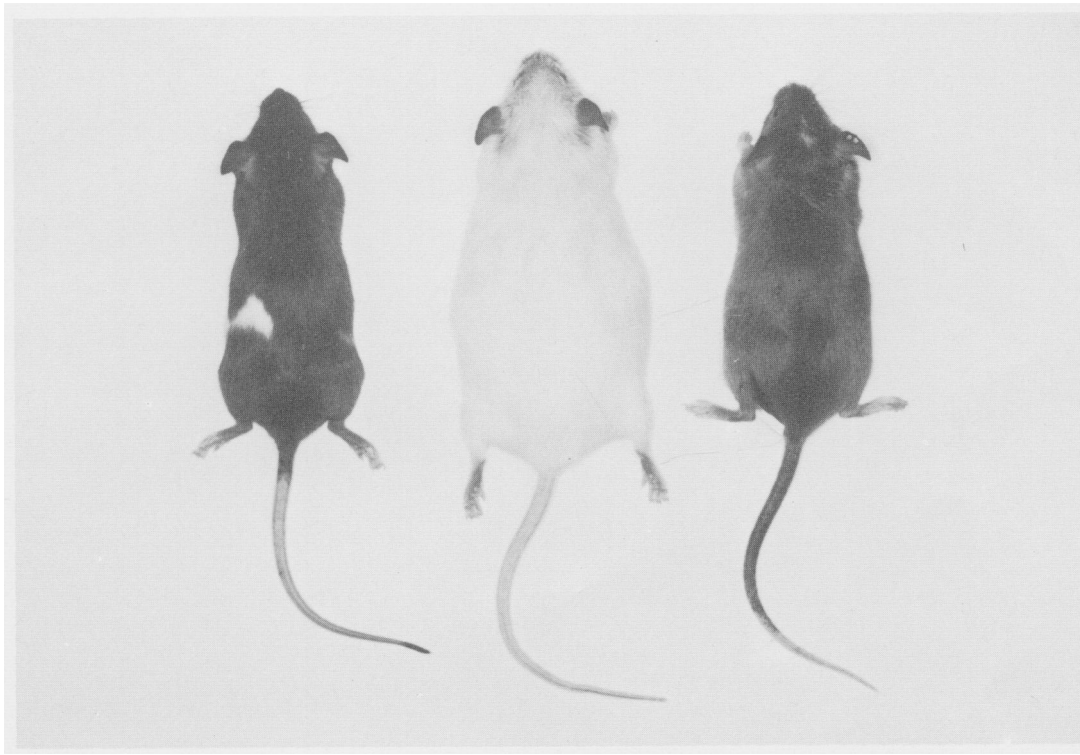

PLATE 1

$W^{ct}+/+Ph$ multiparous female mouse aged 10 months flanked on left by $Ph/+$ virgin female aged 2 months and on right by $W^{ct}/+$ virgin female also 2 months. Pigment in $W^{ct}+/+Ph$ is dilute and confined to ears and muzzle. $Ph/+$ is full agouti with white belly patch extending in this individual to flanks, spotted tail with black tip and white paws. $W^{ct}/+$ is slightly less full agouti with frontal blaze and spotted tail: there are a few scattered white hairs barely visible in the illustration. $W^{ct}/+$ spotting may extend over parts of the body.

White cell counts were made similarly on mice aged 2 months or more and are recorded in Table 3. The counts, total and differential, of $W^{ct}/+$ did not differ significantly from those of $+/+$ except those for monocytes where the P value was at best marginal. The mutant $W^{sh}/+$ gave similar results on analysis. $Ph/+$ mice, however, showed an increase, nearly significant ($P < 0{\cdot}10$) but not impressive, in total leucocytes which was attributable to lymphocytes. On the other hand in the two sets of double heterozygote, $W^{ct}+/+Ph$ and $W^{sh}+/+Ph$, the increase in total leucocyte count was more persuasive especially in $W^{ct}+/+Ph$ ($0{\cdot}002 < P < 0{\cdot}02$): all constituents were involved with the greatest significance attaching to polymorphonuclear granulocytes.

Table 3. *Mean values* ($\pm$ *standard error*) $\times 10^{-6}$ *ml for peripheral blood white cells of mice of various genotypes*

Genotype	No. of mice	Total WBC	Polymorpho-nuclears	Lymphocytes	Monocytes
(1) $+/+$	11	6·609	1·155	4·982	0·473
		($\pm$0·448)	($\pm$0·118)	($\pm$0·394)	($\pm$0·041)
(2) $Ph/+$	14	9·050	1·400	7·129	0·521
		($\pm$0·800)	($\pm$0·157)	($\pm$0708)	($\pm$0·091)
(3) $W^{ct}/+$	10	8·240	1·380	6·183	0·680
		($\pm$0·724)	($\pm$0·227)	($\pm$0·544)	($\pm$0·083)
(4) $W^{sh}/+$	11	8·255	1·418	6·182	0·655
		($\pm$0·744)	($\pm$0·190)	($\pm$0·542)	($\pm$0·077)
(5) $W^{ct}+/+Ph$	12	13·125	3·608	8·758	0·742
		($\pm$1·242)	($\pm$0·899)	($\pm$0·648)	($\pm$0·153)
(6) $W^{sh}+/+Ph$	10	12·750	2·950	8·820	0·950
		($\pm$1·383)	($\pm$0·611)	($\pm$0·731)	($\pm$0·218)
P values					
2 *v.* 1		$0{\cdot}05 < P < 0{\cdot}10$	$> 0{\cdot}10$	$0{\cdot}05 < P < 0{\cdot}10$	$> 0{\cdot}10$
3 *v.* 1		$> 0{\cdot}10$	$> 0{\cdot}10$	$> 0{\cdot}10$	$= 0{\cdot}05$
4 *v.* 1		$> 0{\cdot}10$	$> 0{\cdot}10$	$> 0{\cdot}10$	$0{\cdot}05 < P < 0{\cdot}10$
5 *v.* 2		$0{\cdot}002 < P < 0{\cdot}02$	$0{\cdot}002 < P < 0{\cdot}02$	$0{\cdot}05 < P < 0{\cdot}10$	$> 0{\cdot}10$
6 *v.* 2		$0{\cdot}02 < P < 0{\cdot}05$	$0{\cdot}002 < P < 0{\cdot}05$	$0{\cdot}05 < P < 0{\cdot}10$	$> 0{\cdot}10$

Dose–response to whole body X-irradiation. Normally $+/+$ mice of the stock have a MLD of $\sim$ 8 Gy (Loutit, Corp & Adams, 1982). Under similar conditions the responses of $W^{ct}/+$ and $W^{ct}+/+Ph$ were as in Table 4. The MLD were respectively $6{\cdot}49 \pm 0.28$ Gy and $4{\cdot}59 \pm 0{\cdot}14$ Gy, a highly significant difference ($P = 1{\cdot}9 \times 10^{-9}$).

Haematopoietic response to X-irradiation. Blood counts were obtained at 10, 15, 20, 25 and 30 days from $W^{ct}/+$ and W^{ct}/W^{sh} mice after 5 Gy and from $W^{ct}+/+Ph$ after 4 Gy to the whole body together with control $+/+$ or $W^{sh}/+$ mice. The results are illustrated in Figs. 1–3. $W^{ct}/+$ responded to 5 Gy in similar fashion to other W allele heterozygotes (unpublished observations), W^{ct}/W^{sh} more dramatically but also like compounds of other W mutants with W^{sh}. In each case recovery of leucocyte count from the lowest value at 10 days preceded that of red cell count: recovery of RBC began after 15 days in $+/+$ and $W^{sh}/+$ and later in $W^{ct}/+$ and W^{ct}/W^{sh}. There was no mortality in any of the genotypes. $W^{ct}+/+Ph$ also like other double heterozygotes responded more vigorously when tested at the lower dose of 4 Gy: the illustrated figures for the double heterozygote

Table 4. *Mortality of* $W^{ct}/+$ *and* $W^{ct}+/+Ph$ *mice within 30 days after various doses of whole body X-irradiation*

	Mortality after X-ray dose – Gy						
Genotype	3·5	4	4·5	5	6	6·5	7
$W^{ct}/+$	—	0/1	1/14	1/9	4/15	4/8	6/8
$W^{ct}+/+Ph$	0/5	4/14	8/19	12/17	—	—	—

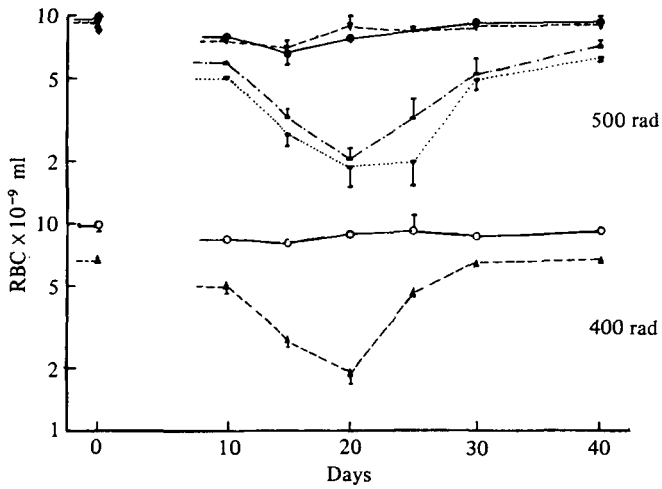

Fig. 1. Red cell counts $\times 10^{-9}$ ml at intervals (days) after total body X-irradiation with 5 Gy (500 rad, top panel) or 4 Gy (400 rad, bottom panel. ●——●, +/+ −5 Gy; ○——○, (+/+ and $W^{sh}/+$) −4 Gy; ▼---▼, $W^{sh}/+$ −5 Gy; ◓—·—◓, $W^{ct}/+$ −5 Gy; ◒·····◒, W^{ct}/W^{sh} −5 Gy; ▲----▲, $W^{ct}+/+Ph$ −4 Gy. Where 1 S.E.M. exceeds the dimension of the plotted point this is indicated by a bar, + or −. Values for untreated animals are shown on the *y* axis.

P values: 5 Gy	10 days	15 days	20 days	25 days	30 days
+/+ *v.* $W^{sh}/+$	0·62	0·82	0·31	—	0·24
+/+ *v.* $W^{ct}/+$	0·024	0·0043	0·0043	—	0·024
+/+ *v.* W^{ct}/W^{sh}	0·10	0·016	0·016	—	0·057
4 Gy					
(+/+, $W^{sh}/+$) *v.* $W^{ct}+/+Ph$	0·004	0·008	0·002	0·072	0·006

are biased on the high side as they represent survivors in the later periods, half the mice having died earlier.

Differential white cell counts revealed (Fig. 3) that until 20 days the polymorphonuclear cells of the mutants were 4 or more times lower than in the controls – indeed in the decade below those plotted in Fig. 3. The counts were so low, virtually unreadable, that the means and standard errors have no real meaning. By 25–30 days the counts had returned to pretreatment levels. In two control groups +/+ and mixed +/+, $W^{sh}/+$, polymorphonuclear cells had not fallen so drastically by 10 days whence they rose progressively to normal or near normal levels. With lymphocytes, however, the values rose from lowest levels at 10 days to about half normal at 30 days, significantly earlier in the controls than in the mutants, i.e.

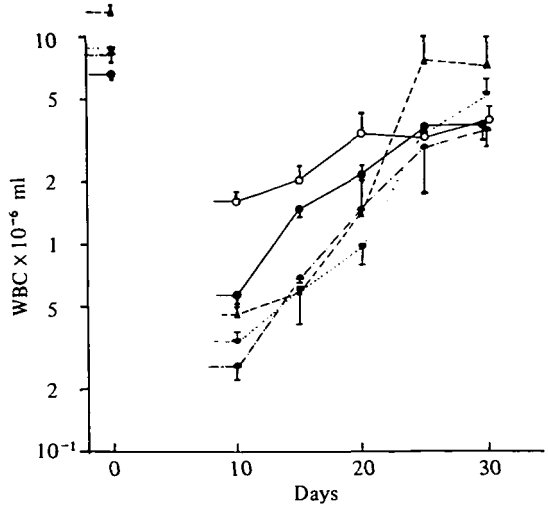

Fig. 2. White cell counts × 10^{-6} ml at intervals (days) after total body X-irradiation with 5 or 4 Gy. ●——●, +/+ −5 Gy; ○——○ (+/+ and $W^{sh}/+$) −4 Gy; ◓—·—◓, $W^{ct}/+$ −5 Gy; ◒·····◒, W^{ct}/W^{sh} −5 Gy; ▲----▲, $W^{ct}+/+Ph$ −4 Gy. Where 1 S.E.M. exceeds the dimension of the plotted point this is indicated by a bar, + or −. Values for untreated animals are shown on the *y* axis.

	10 days	15 days	20 days	25 days	30 days
P values:			5 Gy		
+/+ *v.* $W^{ct}/+$	0·024	0·0043	0·21	0·57	0·79
+/+ *v.* W^{ct}/W^{sh}	0·10	0·016	0·15	0·80	0·19
+/+ *v.* $W^{sh}/+$ (not plotted)	0·31	0·88	0·92	0·33	0·17
			4 Gy		
(+/+ and $W^{sh}/+$) *v.* $W^{ct}+/+Ph$	0·004	0·012	0·024	0·29	0·20

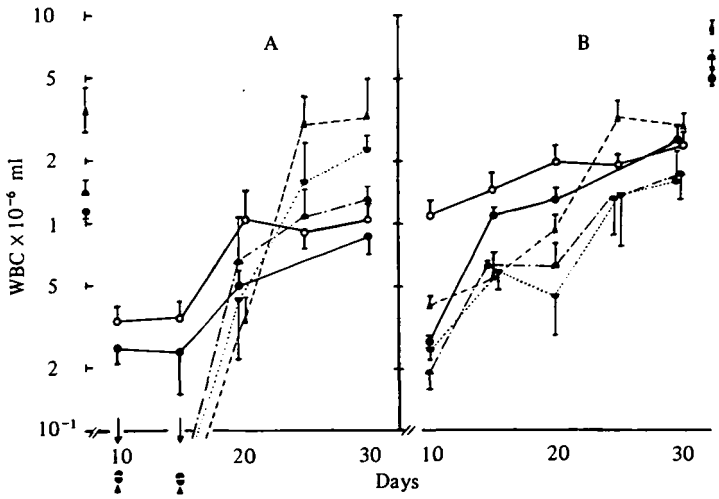

Fig. 3. Polymorphonuclear cells (A) and lymphocytes (B) × 10^{-6} ml at intervals (days) after total body X-irradiation with 5 or 4 Gy. ●——●, +/+ −5 Gy; ○——○ (+/+ and $W^{sh}/+$) −4 Gy; ◓—·—◓, $W^{ct}/+$ −5 Gy; ◒·····◒, W^{ct}/W^{sh} −5 Gy; ▲----▲, $W^{ct}+/+Ph$ −4 Gy. The bar, + or −, indicates 1 S.E.M. Values for untreated animals are shown on the *y* axis, left in (A), right in (B).

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the total white cell counts (Fig. 2) are all largely expression of the major lymphocyte component.

Affirmation with W^e+ *and* $W^e+/+Ph$. Earlier observations on $W^e/+$ and $W^e+/+Ph$ had indicated that *Ph* had influence on the blood count and radiosensitivity but had been less dramatic than with W^{ct}. Individual $W^e/+$ mice vary from mild erythropenia to mild erythrocytosis (cf. Geissler *et al.* on variation between genotypes). The result is a normal mean (see Table 5) for red cell count and red cell volume. $W^e+/+Ph$ differ with a proportion of moderately anaemics: thus the mean value for RBC is reduced and the mean corpuscular volume higher. Six weeks after near-lethal whole body irradiation the pre-irradiation mean values for red cells had been attained with somewhat higher cell size. There was no mortality in these groups.

Table 5. *Mean values* (± *standard error*) *for peripheral blood red cells of mice, genotypes* $W^e/+$ *and* $W^e+/+Ph$

Genotype	*n*	RBC ($\times 10^{-9}$ ml)	PCV (%)	MCV (μm^3)	
$W^e/+$	10	9·95 (±0·37)	45·95 (±1·2)	46·5 (±1·3)	Untreated
$W^e+/+Ph$	25	8·08 (±0·33)	41·3 (±1·2)	51·9 (±1·2)	Untreated
P value of difference		0·0041	0·0020	0·014	
$W^e/+$	15	9·83 (±0·24)	48·3 (±0·67)	49·5 (±1·1)	6 weeks after X-irradiation 5 and 4·5 Gy – data aggregated (MLD's 6 and 5 Gy respectively)
$W^e+/+Ph$	11	8·06 (±0·31)	44·5 (±0·73)	55·9 (±1·9)	6 weeks after X-irradiation 5 and 4·5 Gy – data aggregated (MLD's 6 and 5 Gy respectively)
P value of difference		< 0·002	< 0·002	0·002 < *P* < 0·02	

4. DISCUSSION

(1) W^{ct}. The mode of inheritance of the observed spotting in the cross to wild type +/+ (Table 1 (1)) is that of an autosomal dominant. Further observations from the intercross (Table 1 (2)) are consistent with the thesis of autosomal dominance and with the new mutation W^{ct} being an allele at the *W* locus: shortage of homozygotes indicates pre-natal losses, common when each *W* locus is occupied by mutant alleles. The data on *Rw* combination and linkage (Table 1 (3) and (4)) provide additional evidence that W^{ct} is in fact a *W* allele. Absence of double mutants and of wild-type progeny in Table 1 (4) indicates the close linkage of W^{ct} and *Rw*.

The additional information derived by producing compounds with other *W* mutations and with *Ph* (Table 1 (5)–(11)) are consistent with W^{ct} being an allele at the *W* locus. The compound with W^{sh} which is an exceptional allele in that the homozygote is not only fully viable but fertile and non-anaemic (Lyon & Glenister, 1982) was, as anticipated, fertile. The compound with W^v was unexpectedly fertile but weakly and anaemic and that with W^e also anaemic and still more weakly:

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the fertility of the latter was not tested. The double heterozygote of W^{ct} and *Ph* was of interest particularly in respect to its anaemia.

(2) *Haematology*. Heterozygotes of W^* mutants have mean red cell counts normal or slightly elevated or slightly reduced (Geissler *et al.* 1981). W^v is the classic and oldest established example of the last category. $W^{ct}/+$ is similar and the mean corpuscular volume, as in $W^v/+$, is slightly raised. $W^e/+$ has values in the normal range. Nevertheless as double heterozygotes with *Ph* both W^{ct} and W^e show anaemia and macrocytosis, substantial in the case of $W^{ct}+/+Ph$. This is more than the modest change first noted by Grüneberg & Truslove (1960) for $W^v+/+Ph$ and supported by observations in Harwell mice (Table 2).

The anaemia and macrocytosis of W^v mice are attributed to defective erythropoiesis of unknown causation but associated with impaired response to erythropoietin but not to anoxia (Russell, 1979). The exaggeration of the mild anaemia in $W^{ct}/+$ and $W^v/+$ and the revelation of anaemia in $W^e+/+Ph$ when heterozygous with *Ph* point to a role of *Ph* exaggerating an erythropoietic defect in most W^* mutants.

The presence of an effect of *Ph* on total haemotopoiesis is also suggested by the leucocyte counts of untreated animals bearing *Ph* gene. Between inbred strains of mice mean leucocyte counts vary (Russell & Bernstein, 1966), so that genetic constitution does have an effect. In the current set of non-inbred mice, though one would expect variation between individuals, the notable feature was the consistently high leucocyte counts of mice with the *Ph* gene.

The exaggerated response of $W^*/+$ (and still more of W^*/W^* mice) to whole body irradiation resulting in death from the 'radiation syndrome' with pancytopenia of the cells and formed elements of the blood must be more than a failure of erythropoiesis (Fig. 1). It reveals additionally weakness of the pluripotent stem cell. As a result there is delayed return of leucocytes to the peripheral blood (Fig. 2) and microvascular incompetence with haemorrhage at doses of X-rays withstood by normal mice. The further exaggeration in double heterozygotes with *Ph* (Loutit *et al.* 1982) is demonstrated again with the marked reduction ($\sim$ 2 Gy) of MLD when $W^{ct}+/+Ph$ is compared with $W^{ct}/+$. Thus *Ph* must complement W^* not only in erythropoiesis but in the various functions of the pluripotent stem cell.

Russell *et al.* (1963) attributed the extreme radiosensitivity of W/W^v mice 'very largely to their differential rates of regeneration of erythropoietic tissue'. Their demonstration of delayed recovery of marrow cellularity was convincing. However, in our different genotypes there is delay also in delivery of leucocytes to peripheral blood (Fig. 2), but when recovery does begin the rate of increase of both red corpuscles and leucocytes is virtually as rapid as in $+/+$ mice. It is a common experience with irradiated mice that after some preliminary hesitations mean leucocyte counts rebound to a sub-normal plateau after about 4 weeks but that it may take 6 months or more for the lymphocytic component ultimately to reach normal levels. Possibly the same may apply for red corpuscles in certain mice with underpriveleged marrow such as ours.

Delay in recovery is one factor in accounting for mortality; increased radiosensitivity is another. A standard method of assessing radiosensitivity, measuring D_0 of colony forming units in spleen (CFUs), is not applicable, as W/W^v have been

shown to be grossly deficient in CFUs (McCulloch *et al.* 1964) and we find smaller defects in all mice with *W* mutations (to be published). Nevertheless W/W^v and analogues are but slightly defective in haematopoietic stem cells according to the ability of their marrow to restore lethally irradiated mice (Harrison, 1972; Loutit *et al.* 1981). *Pro tem* we note that the observed data suggest increased radiosensitivity, e.g. the granulocyte count of *W** mutants falls virtually to zero and remains there for more than 2 weeks after doses of X-rays (4–6 Gy) that have marked but non-lethal effects on normal mice (Fig. 3). These doses of X-rays are associated in the mutant mice with haemorrhage such as one sees in the platelet-microvascular disorder of the lethal radiation syndrome: a consequent inapparent leak of red corpuscles into extra-vascular water would account for the exaggerated anaemia of these mice.

5. CONCLUSION

The deduction from the present work is that the control on haematopoiesis exerted by Chromosome 5 is not confined solely to the *W* locus but extends at least to *Ph* and probably to *Rw* (preliminary unpublished data). Hitherto what has received most attention is the defect in *W** mutants, particularly double mutants, of erythropoiesis to give anaemia and delay in recovery from irradiation (Russell *et al.* 1963). It is now shown that *Ph* has (1) a natural influence on the numbers of circulating leucocytes, (2) a collaborative action on erythropoiesis in conjunction with *W** mutants, especially W^{ct} and (3) a significant effect on response to X-irradiation, specially notable in conjunction with W^{ct}, a difference of 2 Gy in median lethal dose. We consider that (3) is an indication of fault in the pluripotent haemoatopoietic stem cell as there is circumstantial evidence of involvement of all major constituents of peripheral blood.

We are specially indebted to Mr D. Papworth for statistical analysis and also to Mr G. Wilkins for photography, Mr M. Corp and Mr P. Adams for X-irradiation and Miss D. Walker for the typescript. J.F.L. thanks the M.R.C. for a grant to pursue this work.

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