Genet. Res., Camb. (1982), **39**, *pp.* 275–285
Printed in Great Britain

Genetic basis of susceptibility to splenic lipofuscinosis in mice

By D. N. CRICHTON and J. G. M. SHIRE*†

*MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh EH4 2XU, Scotland, * Institute of Genetics, University of Glasgow, Glasgow G11 5JS, Scotland, and † Department of Biology, University of Essex, Colchester CO4 3SQ, England*

(*Received 5 October 1981 and in revised form 12 November 1981*)

SUMMARY

Spleens with black pigment in them were found in 4–57 % of mice from 17 stocks, all sublines of C57BL or with significant C57BL ancestry. Splenic lipofuscinosis was absent from 16 stocks, including three C57BL/6By × BALB/cBy recombinant – inbred lines. Progeny testing showed all C57BL/6J mice to be equally likely to develop black spleens. The penetrance of lipofuscinosis differed between sublines but not between the sexes or between laboratories. Susceptibility to lipofuscinosis showed dominant, autosomal, inheritance in F1 hybrids. Observations on backcrosses and on recombinant inbred lines and their intercrosses indicated the existence of two genetic factors. Splenic lipofuscinosis was prevented either by a deficiency of melanin or by homozygosity for a non-C57BL allele close to the *c–p* region of chromosome 7. The presence of a C57BL allele at a locus on another chromosome is necessary for lipofuscinosis to occur.

1. INTRODUCTION

Blackening of the anterior end of the spleen has been shown to occur in some, but not all, mice from colonies of C57BL mice (Crichton *et al.* 1978*a*). The appearance and histochemical properties of the pigment suggest that it belongs to the lipofuscin group of autogenous pigments (Pearse, 1972; Crichton, Busuttil & Price, 1978*b*; Crichton, Busuttil & Ross, 1980). Lipofuscins usually accumulate in ageing tissues but the pigment found in the spleens of C57BL mice is present in animals only three weeks old (Crichton *et al.* 1978*a*).

This paper describes investigations of the inheritance of susceptibility to splenic lipofuscinosis using hybrid, congenic and recombinant–inbred mice. Particular attention was paid to comparisons between amelanotic albino mice and mice able to synthesize melanin as there is controversy about the relationship between lipofuscin and melanin and about the exact chemical composition of lipofuscin (Pearse, 1972).

† Present address: Department of Biology, University of Essex, Colchester CO4 3SQ England.

2. MATERIALS AND METHODS

Mice of 32 different stocks and 19 hybrid crosses were raised in Edinburgh, Glasgow and in Colchester. Details of the stocks and crosses are given in Tables 1–7. The seven recombinant inbred lines, CXBD/By, CXBE/By, CDBG/By, CXBH/By, CXBI/By, CXBJ/By and CXBK/By, derived from intercrossing BALB/cBy and C57BL/6By mice (Bailey, 1971; Swank & Bailey, 1973), raised at the University of Essex were descended, via colonies in Glasgow and at Carshalton, from the original Jackson Laboratory lines. C57BL/10ScSn mice bred in Hull were purchased from Bantin & Kingman Ltd. and examined in Edinburgh. Two lines, one segregating at the albino (*c*) locus and the other segregating at the pink-eyed dilution (*p*) locus, were kindly supplied by Dr M. F. W. Festing of the MRC Laboratory Animal Centre, Carshalton. These incipient congenic lines represented the seventeenth backcross of the mutations to the C57BL/10ScSn background.

All mice were bred and maintained under controlled conditions and allowed free access to food and water. The mice were fed a standard rodent diet (Spratt's Laboratory Animal Services, Barking, Essex) in Edinburgh, Oxoid Breeding Diet in Glasgow and BP Expanded Rat & Mouse Breeder Diet No. 3 in Essex. Spleens were examined at autopsy and the presence or absence of pigmentation recorded. When there was any uncertainty spleens were placed in cold water and refrigerated for a day to clear the tissue of haemoglobin. There proved to be no difficulty in distinguishing between affected and unaffected individuals within an affected strain. Laparotomy was carried out under tribromoethanol anaesthesia in Edinburgh. The laparotomised mice were mated and the phenotype of their offspring recorded.

Statistical comparisons were made with the χ^2 test.

3. RESULTS

Lipofuscinosis of the spleen occurred in all the five sublines of C57BL mice examined (Table 1) and in eight stocks derived from C57BL (Table 2). No significant sex-differences in the frequency of black spleens, even at the 0·05 probability level, have been found for any of the stocks in Tables 1 and 2 or for any of the stocks in Tables 4–7. A non-parametric signed-pairs test for 21 comparisons of males and females gave $\chi^2_{(1)} = 0{\cdot}5$. No significant heterogeneity in the incidence of lipofuscinosis was found when observations on C57BL/10ScSn mice from four sources were compared. The samples of C57BL/6By mice examined in Glasgow and in Essex did not differ significantly in the frequency of occurrence of black spleens. Significant differences were found in the incidence of lipofuscinosis between the four C57BL sublines reared in Edinburgh ($\chi^2_{(3)} = 27$, $P < 0{\cdot}001$) but not between the two sublines in Essex or between the small samples of three sublines in Glasgow. Significant heterogeneity was found in the incidence of black

Table 1. *Occurrence of splenic lipofuscinosis in male and female C57BL mice reared in different places*

Subline	Place	Sex	Age (wks)	No affected	Total	Incidence (%)
C57BL/10ScSn	Edinburgh	M	6–20	31	129	24
		F	6–20	20	78	26
	Essex	M	7–17	9	27	35
		F	8–16	5	20	20
	Glasgow	F	24–38	2	20	10
	Hull	M & F	8–12	9	52	17
C57BL/6By	Essex	M	8–13	5	42	12
		F	8–15	9	32	27
	Glasgow	M	10–12	2	7	29
		F	12–19	2	13	15
C57BL/6J	Edinburgh	M	3–26	58	197	29
		F	3–12	49	141	35
C57BL/Fa	Edinburgh	M	4–10	11	110	10
		F	4–12	1	27	4
C57BL/Tb	Glasgow	M	12–26	5	16	31
		F	9–48	3	12	25

Table 2. *Occurrence of splenic lipofuscinosis in stocks derived from C57BL inbred strains*

Stock	Place	Sex	Age (wks)	Number affected	Total	Incidence (%)
C57BL/Wr congenic for *Wr*	Edinburgh	M	5–12	22	84	26
		F	9	7	59	12
C57BL/Wr × C57BL/6J F1	Edinburgh	M	3	3	23	13
		F	3	3	22	14
C57BL/6J-dy congenic for *dy*	Glasgow	M	8–30	10	20	50
		F	8–30	20	33	60
B10.BR congenic for *H-2^k*	Essex	M	10–18	3	49	6
		F	8–18	5	26	19
C57BL/10ScSn congenic for *c*	Essex	M	8–16	7	43*	16
		F	8–13	15	47*	32
C57BL/10ScSn congenic for *p*	Essex	M	8–12	16	55*	29
		F	8–13	8	58*	14
C57BL/Tb × C57BL/10ScSn F1	Essex	M	8–14	14	48	28
		F	8–13	17	51	34
Q (C57BL/Fa ancestry)	Edinburgh	M	15	3	76	4
		F	19	1	23	4

* Excludes non-pigmented mice, see Table 8.

https://doi.org/10.1017/S0016672300020954 Published online by Cambridge University Press

spleens amongst the lines derived from C57BL, both in Edinburgh ($\chi^2_{(1)} = 13{\cdot}1$, $P < 0{\cdot}01$) and in Essex ($\chi^2_{(3)} = 10{\cdot}4$, $P < 0{\cdot}02$).

There was significant heterogeneity within the groups of stocks raised in Edinburgh, Essex and Glasgow when both sublines and derived stocks were considered. The values of χ^2 were 55 ($P \ll 0{\cdot}001$), 12·3 ($P < 0{\cdot}05$) and 18·4 ($P < 0{\cdot}001$). The highest incidence found was 57 % in the C57BL/6J-*dy* stock in Glasgow and the lowest was 4 % in the Q stock in Edinburgh. Table 3 shows ten

Table 3. *Stocks in which lipofuscinosis was not found*

Stock	Place	Sex	Age (wks)	Number examined	Overall incidence	Coat colour
BALB/c	Edinburgh	M	9–13	10	0/51	Albino
		F	9–21	41		
BALB/cBy	Essex	M	8–15	55	0/131	—
		F	8–15	76		
	Glasgow	M	12–18	14	0/20	—
		F	12–29	6		
CBA/Ca	Edinburgh	M	8–39	12	0/33	Agouti
		F	7–39	21		
CBA/FaCam	Glasgow	M	9–30	25	0/45	—
		F	6–36	20		
C3H/He	Edinburgh	M	7–19	20	0/42	Agouti
		F	7–40	22		
DBA/2J	Glasgow	M	5–46	23	0/31	Dilute brown
		F	10–30	8		
129/ReJ	Edinburgh	M	9	8	0/16	Pale brown
		F	12–20	8		
BH	Edinburgh	M	8	7	0/26	Light brown with white patches
		F	8–10	19		
VM	Edinburgh	M	12–13	16	0/25	Albino
		F	12	9		
CRCD	Edinburgh	M	19	10	0/18	Albino
		F	13–19	8		
Peru	Glasgow	M	25–41	11	0/29	Agouti
		F	20–50	18		
Porton	Edinburgh	M	7–9	29	0/67	Mixed
		F	7–10	38		
C3H × CBA/Ca F1	Edinburgh	M	3	39	0/67	Agouti
		F	3	28		

strains, including two sublines of BALB/c and CBA, and one F1 hybrid in which lipofuscinosis of the spleen did not occur.

Matings were set up between C57BL/6J mice of known phenotype, determined by laparotomy, to test for genetic heterogeneity in susceptibility to lipofuscinosis within this highly inbred stock. The incidence of lipofuscinosis did not differ significantly ($\chi^2_{(3)} = 5{\cdot}1$) between the four groups of offspring in Table 4. It was independent of whether one or other, both or neither of the parents had black spleens.

C57BL mice of the 6J, 6By and Tb substrains were mated to mice of seven different stocks, none of which showed splenic lipofuscinosis. Some male F1 mice with black spleens were found in every outcross of each of the three kinds of C57BL males (Table 5). Females with lipofuscinosis were found in each of the F1 crosses

Table 4. *Incidence of lipofuscinosis amongst offspring of C57BL/6J mice of known splenic phenotype*

Mating	Sex	Number affected	Total	Incidence (%)
1. Pigmented × pigmented	M	27	82	33
	F	27	73	37
2. Pigmented × nonpigmented	M	18	37	49
	F	11	21	52
3. Nonpigmented × nonpigmented	M	7	17	41
	F	8	17	47
4. Matings of cross 1 progeny	M	31	70	44
	F	24	53	45

Table 5. *Incidence of lipofuscinosis in the spleens of F1 mice from crosses between affected and unaffected strains*

Cross	Place	Sex	Age (wks)	Number affected	Total	Incidence (%)	
C57BL/6By × BALB/cBy	Essex	M	8–16	9	43	21	27
		F	8–16	12	34	35	
	Glasgow	M	7–9	2	8	25	
BALB/cBy × C57BL/6By	Essex	M	8–14	15	52	29	33
		F	8–14	21	57	37	
	Glasgow	M	6–7	1	8	13	
C57BL/6J × BALB/c*	Edinburgh	M	3–4	19	42	45	43
		F	3–4	16	40	40	
BH × C57BL/6J	Edinburgh	M	3–4	3	34	9	10
		F	3–4	4	34	12	
C3H/He × C57BL/6J	Edinburgh	M	3–4	3	14	21	19
		F	3–4	2	12	17	
129/ReJ × C57BL/6J	Edinburgh	M	3–4	4	9	44	35
		F	3–4	3	11	27	
VM × C57BL/6J	Edinburgh	M	3–4	2	45	4	6
		F	3–4	3	43	7	
DBA/2J × C57BL/Tb	Glasgow	M	20	1	2	50	25
		F	18–20	0	3	0	
C57BL/Tb × DBA/2J	Glasgow	F	26	1	9	11	

* Reciprocals combined.

in which more than three females were scored. No significant differences in the incidence of lipofuscinosis were found between males and females of the same F1 cross, or between reciprocal crosses between C57BL/6By and BALB/cBy. A significant difference ($\chi^2_{(1)} = 9{\cdot}8, P < 0{\cdot}01$) was found between the overall incidence in the BALB/cBy × C57BL/6By F1 mice in Essex and the BALB/c × C57BL/6J

https://doi.org/10.1017/S0016672300020954 Published online by Cambridge University Press

F1 mice in Edinburgh. Significant heterogeneity ($\chi^2_{(4)} = 43$, $P \ll 0{\cdot}001$) was found amongst the five different F1 crosses were made with C57BL/6J males in Edinburgh (Table 5).

The frequencies of occurrence of black spleens in male and female mice of the CXB recombinant–inbred lines are shown in Table 6. Significant heterogeneity was found amongst the seven lines studied at Essex ($\chi^2_{(6)} = 170$, $P \ll 0{\cdot}001$) and

Table 6. *Occurrence of lipofuscinosis in mice of the C57BL/6By × BALB/cBy recombinant inbred strains and their intercrosses*

CXB line	Sex	Number affected	Total	Incidence (%)	Coat colour
D	M	3	52	6	Agouti *AABBCC*
	F	4	54	7	
	*M & F	6	37	16	
E	M	8	41	20	Agouti *AABBCC*
	F	11	48	23	
	*M & F	7	41	17	
G	M	0	41	0	Albino *AAbbcc*
	F	0	39	0	
	*M & F	0	36	0	
H	M	24	43	56	Brown *aabbCC*
	F	20	46	43	
	*M & F	7	15	47	
I	M	0	41	0	Albino *aabbcc*
	F	0	57	0	
	*M & F	0	9	0	
J	M	0	47	0	Brown *aabbCC*
	F	0	48	0	
K	M	2	36	6	Black *aaBBCC*
	F	2	33	6	
	*M & F	2	18	11	
I × J F1	M	0	38	0	Brown *aabbCc*
	F	0	35	0	
G × J F1	M	6	66	9	Brown agouti *AabbCc*
	F	4	73	5	
G × I F1	M	0	20	0	Albino *Aabbcc*
	F	0	28	0	

All mice at Essex were 8–13 weeks old.
* Data from mice raised in Glasgow, 8–25 weeks old.

amongst the six lines observed in Glasgow ($\chi^2_{(5)} = 21$, $P < 0{\cdot}001$). Lipofuscinosis was absent from three lines (CXBG, CXBI and CXBJ) and present in four. The four positive lines had incidences ranging from 6 to 50%. Heterogeneity tests on these four lines were highly significant ($\chi^2_{(3)} = 66$, $P \ll 0{\cdot}001$). All the positive lines have pigmented fur. Two of the three negative lines are albino, but mice of the third line, CXBJ, have brown non-agouti coats. The three negative recombinant–inbred lines were crossed and their F1 offspring examined (Table 6). Lipofuscinosis was absent from the offspring of crosses between CXBI and both CXBG and CXBJ.

Black spleens were, however, found in about 8% of the offspring of matings between CXBG and CXBJ mice, implying that complementation was occurring.

Backcrosses of C57BL × BALB F1 mice to the parental strain with the recessive, negative, phenotype were made in both Edinburgh and Essex (Table 7). All four possible backcrosses of C57BL/6By to BALB/cBy were bred. The overall incidence

Table 7. *Splenic lipofuscinosis in segregating crosses involving C57BL mice*

Stock	Place	Age (wks)	Sex	No. affected +	No. affected cc	Incidence (%) +	Incidence (%) cc
BALB/cBy × (BALB/cBy × C57BL/6By)	Essex	8–13	M	4/19	0/14	21	0
			F	1/9	0/11	11	0
BALB × (C57BL × BALB)	Essex	8–13	M	4/27	0/22	15	0
			F	2/26	0/23	8	0
(BALB × C57BL) × BALB	Essex	8–13	M	6/34	0/24	18	0
			F	4/30	0/31	13	0
(C57BL × BALB) × BALB	Essex	8–13	M	0/20	0/16	0	0
			F	3/24	0/18	13	0
Combined backcross to BALB/cBy	Essex	—	M	14/100	0/74	14	0
			F	10/89	0/70	11	0
(C57BL/6J × BALB/c) × BALB/c	Edinburgh	3	M & F	10/47	0/62	21	0
C57BL × (C57BL × BALB)	Edinburgh	3	M & F	10/76	0/0	13	0
(C57BL × BALB) F2	Edinburgh	3	M & F	22/81	0/37	27	0
(C57BL/Tb × DBA/2J) × DBA/2J	Glasgow	16–28	M	7/46		(15)	
		5–8	F	2/30		(7)	

Table 8. *Incidence of lipofuscinosis in stocks, nearly congenic with C57BL/10ScSn, segregating at the albino or pink-eyed dilution loci*

Stock C	Sex	Age (wks)	Affected	Total	Incidence (%)
Non-albinos	M	8–16	7	43	16
	F	8–13	15	47	32
Albinos	M	8–13	0	11	0
	F	8–13	0	6	0
Stock P					
Non-pinkeyed	M	8–13	16	55	29
	F	8–13	8	58	14
Pinkeyed	M	8–13	0	26	0
	F	8–13	0	39	0

of lipofuscinosis was 7·8% and did not differ significantly between the four backcrosses. The incidence in the corresponding F1 mice was 28%. Similarly in Edinburgh the overall incidence amongst the C57BL/6J backcross to BALB/c hybrids was about a quarter (9·2%) of the incidence in the corresponding F1 mice (43%). In both places no albino mice were found with black spleens. Contingency χ^2 tests showed significant ($P < 0{\cdot}001$) association of pigmentation of the fur and pigmentation of the spleen. The frequency of lipofuscinosis amongst the pigmented

backcross mice was 12·7% in Essex and 21·2% in Edinburgh: about half the corresponding F1 frequencies. Lipofuscinosis was found amongst backcross to C57BL/6J mice and amongst pigmented F2 mice but not in any of the albino F2 mice (Table 7). The association of lipofuscinosis with pigmented fur was significant ($P < 0·01$) for the F2 mice. Mice with pigmented spleens were also found in the backcross of C57BL/Tb to DBA/2J, a lipofuscin – negative strain. Two stocks, effectively congenic with C57BL/10LScSn and carrying the pigmentation mutations *c* and *p*, were investigated because of the apparent restriction of lipofuscinosis to pigmented mice. Whilst significant numbers of normally pigmented mice had lipofuscinosis, none of their albino or pink-eyed sibs had black spleens (Table 8). The association between the occurrence of lipofuscinosis and strongly pigmented fur was significant for the stock segregating for *p* ($\chi^2_{(1)} = 14·2$, $P < 0·001$) and for the one segregating for *c* ($\chi^2_{(1)} = 3·8$, $P = 0·05$).

4. DISCUSSION

Pigmentation of the spleen was found in seventeen stocks of mice, all sublines of C57BL or with significant C57BL ancestry, and was absent from sixteen other stocks of mice. Pigmentation restricted to a single organ, the liver, has been found in Corriedale sheep and in the Dubin-Johnson syndrome in man. In both cases susceptibility is inherited as an autosomal recessive and the pigment is derived from porphyrins (Engelking & Gronwall, 1979; Swartz, Sarna & Varma, 1979) and is not related to melanin or lipofuscin as had been thought earlier (Pearse, 1972).

Although only some individuals of each affected line of mice had black spleens the observations show susceptibility to be dominantly inherited. The phenotypes of the reciprocal crosses rule out sex-linked and sex-limited genes and show that the condition is not maternally inherited.

The seven recombinant-inbred (RI) lines derived from a cross of the C57BL/6By and BALB/cBy inbred strains fell into two groups: one of four lines in which lipofuscin was deposited in the spleen and the other of three lines that were free of lipofuscinosis. The recovery of both the original phenotypes in a set of only seven recombinants implies that the genetic basis of differences in susceptibility to splenic lipofuscinosis may be fairly simple. In two independent backcrosses of C57BL × BALB F1 mice to the recessive parental strain the incidence of lipofuscinosis was about a quarter of that in the corresponding F1 mice, suggesting segregation at two loci. Further evidence for the existence of two loci comes from the appearance of mice with black spleens in the intercross of two RI lines, CXBG and CXBJ, free of lipofuscinosis. The absence of lipofuscinosis from all albino F2 and backcross mice suggested that this locus might be associated with differences in susceptibility. The incidence of black spleens amongst the pigmented backcross mice was about half that in the corresponding F1 mice, suggesting an epistatic effect of the albino region of chromosome 7 on susceptibility. The findings on the stock effectively congenic with C57BL10/ScSn that was segregating at the albino locus strengthened this association, but could not distinguish between pleiotropy

or linkage as its cause. Although two of the non-susceptible RI lines are albino the third one, CXBJ, is not. CXBJ mice must thus have the non-C57BL allele at the second locus. This allele must be recessive, otherwise the pigmented F1 mice would not be susceptible. If the two alleles at this locus are *Bsp*, found in C57BL, and *bsp*, found in non-susceptible stocks, and if the alleles associated with the albino locus are represented by *C* and *c* then the genotypes of the various stocks are those given in Table 9. All four susceptible RI lines would be *Bsp Bsp, CC*. The pattern of occurrence of the alleles at the postulated black spleen, *Bsp*, locus does not match

Table 9. *Susceptibility to lipofuscinosis and proposed genotypes for six stocks of mice*

	Genotype	Susceptibility
C57BL	*BspBsp CC*	+
BALB	*bspbsp cc*	−
C57BL × BALB F1	*Bspbsp Cc*	+
CXBG	*BspBsp cc*	−
CXBI	*bspbsp cc*	−
CXBJ	*bspbsp CC*	−

exactly any of those recorded for marker genes (Swank & Bailey, 1973; Oliverio, 1979). It is closest to the patterns found for *Ig-1* on chromosome 12, *H-1* on chromosome 7 and *H-18* and *Exa* (open-field exploration) on chromosome 4.

The resistance factor associated with the albino chromosome region of the BALB genome might be the albino locus or a locus linked to it. The finding that none of the congenic mice homozygous for pink-eyed dilution, *p*, had black spleens could be due to the same linked locus or to pleiotropic effects of the *p* allele. Both *c* and *p* homozygotes have severe defects in melanin formation in the skin and eyes. The two loci, *c* and *p*, are linked, 13 units apart on chromosome 7. Observations of a C57BL stock in which spontaneous mutation at the *c* or *p* locus had occurred would distinguish between a resistance gene separate from both *c* and *p* and direct resistance as a pleiotropic consequence of inability to synthesise melanin. If the latter then lipofuscin may be more closely related to melanin than is thought at present (Pearse, 1972).

Ultrastructural studies (Crichton *et al.* 1980) have shown that the pigment in the spleen of C57BL mice is laid down in lysosomes. It is generally agreed that the biogenesis of lipofuscin involves lysosomes (de Duve & Wattiaux, 1966; Miguel *et al.* 1977) whilst melanin deposition in the skin and eye takes place in melanosomes, which appear to be a special class of lysosomes. Several mutations are known that affect both pigment formation in the skin and lysosomal function in the kidney and in leucocytes (Brandt, Elliott & Swank, 1975; Hakansson & Lundin, 1977; Novak & Swank, 1979; Gibb *et al.* 1981). Thus the protection from splenic lipofuscinosis conferred by homozygosity for *c* or *p* might be due either to the reduction or absence of melanin itself or to consequential changes in lysosomal functioning.

The strain distribution pattern for the second locus involved in susceptibility to lipofuscinosis suggests that it might be linked to one of several immunoglobulin or histocompatibility loci. It is possible, since loci for such phenotypes are often clustered (Lundin, 1979), that this second factor alters the immune function of splenic macrophages in C57BL mice so that they respond to an environmental stimulus or stress by laying down pigment. Alternatively the susceptibility factor might affect the course of migration of cells in the fetus. The Steel (*Sl*) and Dominant spotting (*W*) mutations disrupt the normal embryonic migration of pigment cells and haemopoetic cells (Russell, 1979).

While all the C57BL stocks are susceptible to lipofuscinosis its incidence varied significantly between stocks. Even in the stocks with the highest incidence some of the individuals were unaffected. Progeny tests showed that residual genetic heterogeneity was not responsible for the incomplete penetrance of splenic lipofuscinosis, suggesting that pigment deposition only occurs in the spleens of susceptible mice in response to an environmental factor. Possible precipitating factors could include viral infection or stress-responses mediated by corticosteroids, for C57BL mice have particularly well developed adrenal responses to stress (Levine & Treiman, 1964; Doering *et al.* 1972; Shire, 1981). The constancy of incidence within the same genotype in different laboratories and on different diets contrasts with the significant differences in incidence found between different C57BL stocks raised in the same environment. Clearly there are differences between different C57BL stocks and derived lines that affect the interaction of mice with the critical environmental factor, just as there are in other traits (Bailey, 1978; Festing, 1979). The high penetrance of lipofuscinosis in the *dy* congenic line and in the CXBH RI line suggest that genetic factors controlling the degree of penetrance might be identifiable. It is of considerable interest that CXBH mice stand out as being highly susceptible to *Leishmania* infection, in which phagocytosis by macrophages and lysosomal fusion are involved (Blackwell, 1981).

The modes of action of the two loci determining susceptibility to splenic lipofuscinosis are being investigated as are differences in penetrance and possible genetic differences in the extent of pigmentation of individual spleens.

The skilful help of Margaret McKendrick and Rose Ide are gratefully acknowledged.

REFERENCES

Bailey, D. W. (1971). Recombinant inbred strains. An aid to finding the identity, linkage and function of histocompatibility and other genes. *Transplantation* **11**, 325–327.

Bailey, D. W. (1978). Sources of subline divergence and their relative importance for sublines of six major inbred strains. In *Origins of Inbred Mice* (ed. H. C. Morse), pp. 197–215. New York: Academic Press.

Blackwell, J. M. (1981). The role of the house mouse in disease and zoonoses. In *Symposia of the Zoological Society of London, 47, Biology of the House Mouse.* (ed. R. J. Berry), pp. 591–616. London: Academic Press.

Brandt, E. J., Elliott, R. W. & Swank, R. T. (1975). Defective lysosomal enzyme secretion in kidneys of Chediak-Higashi (beige) mice. *Journal of Cell Biology* **67**, 774–778.

CRICHTON, D. N., BUSUTTIL, A., PRICE, W. H., ROBERTSON, J. & SWINTON, J. (1978*a*). Lipofuscin deposition in the spleens of mice. *Journal of the Institute of Animal Technicians* **29**, 1–6.

CRICHTON, D. N., BUSUTTIL, A. & PRICE, W. H. (1978*b*). Splenic lipofuscinosis in mice. *Journal of Pathology* **126**, 113–120.

CRICHTON, D. N., BUSUTTIL, A. & ROSS, A. (1980). An ultrastructural study of murine splenic lipofuscinosis. *Journal of Ultrastructural Research* **72**, 130–140.

DE DUVE, C. & WATTIAUX, R. (1966). Functions of lysosomes. *Annual Review of Physiology* **28**, 435–492.

DOERING, C. H., SHIRE, J. G. M., KESSLER, S. & CLAYTON, R. B. (1972). Cholesterol ester concentration and corticosterone production in adrenals of the C57BL/10 and DBA/2 strains in relation to adrenal lipid depletion. *Endocrinology* **90**, 93–101.

ENGELKING, L. R. & GRONWALL, R. (1979). Bile acid clearance in sheep with hereditary hyperbilirubinemia. *American Journal of Veterinary Research* **40**, 1277–1280.

FESTING, M. F. W. (1979). *Inbred Strains in Biomedical Research* London: Macmillan Press Ltd.

GIBB, S., HAKANSSON, E. M., LUNDIN, L. G. & SHIRE, J. G. M. (1981). Reduced pigmentation (*rp*), a new coat colour gene with effects on kidney lysosomal glycosidases in the mouse. *Genetical Research* **37**, 95–103.

HAKANSSON, E. M. & LUNDIN, L. G. (1977). The effect of a coat colour locus on kidney lysosomal glycosidases in the house mouse. *Biochemical Genetics* **15**, 75–85.

LEVINE, S. & TREIMAN, D. M. (1964). Differential plasma corticosterone response to stress in four inbred strains of mice. *Endocrinology* **75**, 142–144.

LUNDIN, L. G. (1979). Evolutionary conservation of large chromosomal segments reflected in mammalian gene maps. *Clinical Genetics* **16**, 72–81.

MIGUEL, F., ORO, J., BENSCH, K. G. & JOHNSON, J. E. (1977). pp. 133–182 in *Free Radicals in Biology* vol. 3 (ed. W. A. Pryor), New York: Academic Press.

NOVAK, E. T. & SWANK, R. T. (1979). Lysosomal dysfunctions associated with mutations at mouse pigment genes. *Genetics* **92**, 189–204.

OLIVERIO, A. (1979). Uses of recombinant inbred lines. pp. 197–218 in *Quantitative Genetic Variation* (ed. J. N. Thompson and J. M. Thoday), New York: Academic Press.

PEARSE, A. G. E. (1972). *Histochemistry, Theoretical and Applied*, 3rd edn, vol. 2. Edinburgh: Churchill Livingstone.

RUSSELL, E. S. (1979). Hereditary anemias of the mouse: a review for geneticists. *Advances in Genetics* **20**, 358–459.

SHIRE, J. G. M. (1981). Genes and hormones in mice. In *Symposia of the Zoological Society of London 47, Biology of the House Mouse* (ed. R. J. Berry), pp. 547–574. London: Academic Press.

SWANK, R. T. & BAILEY, E. W. (1973). Recombinant inbred lines: value in the genetic analysis of biochemical variants. *Science* **181**, 1249–1251.

SWARTZ, H. M., SARNA, T. & VARMA, R. R. (1979). On the nature and excretion of the hepatic pigment in the Dubin-Johnson syndrome. *Gastroenterology* **76**, 958–964.