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A sex-linked anaemia in the mouse

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INTRODUCTION

The study of abnormal haemoglobins which has lately done so much to deepen our understanding of gene action originated in investigations into inherited anaemias in man. A long series of different inherited anaemias is now also known in the mouse and will no doubt be used for similar studies in the near future. The present paper deals with a new condition which both genetically and haematologically differs from all the others.

ORIGIN, GENETICS AND MORPHOLOGICAL EFFECTS OF SEX-LINKED ANAEMIA

The gene for sex-linked anaemia (proposed symbol *sla*) was discovered by Dr D. S. Falconer in 1958 at Edinburgh, who handed the gene over to this laboratory for haematological study. As Falconer and Isaacson (1962) established that *sla* is a sex-linked recessive gene it was not considered necessary to investigate its genetics further. Consequently, the segregation data given in Table 1 are restricted to only two kinds of matings, the ones necessary for obtaining experimental animals.

Table 1. *Segregation of the gene* sla

Mating type	Number of litters	Born	Number of mice: Normal ♂♂	Anaemic ♂♂	Normal ♀♀	Anaemic ♀♀	χ^2	Litter size
−/*sla* ♂ × +/+♀	21	158	78	0	80	0	—	7·5
−/+ ♂ × +/*sla* ♀	42	310	75	82	153	0	0·347	7·4

Anaemic mice (males hemizygous and females homozygous for the gene) can be easily recognized at birth by their pale colour. But this difference disappears when they are a few days old and they have to be marked in some way if they are to be recognized again. They are smaller than their litter mates at birth, and this difference persists throughout life (Fig. 1, and see also Tables 2 and 3). Among the six segregating litters that were weighed daily from birth to the age of 28 days, there was only one anaemic mouse that, though smaller at birth, recovered after a week and

weighed as much as its normal litter mates. In spite of the smaller size of anaemic mice, their expectancy of life is roughly normal; as for other mutants in the mouse, actual life tables are not yet available.

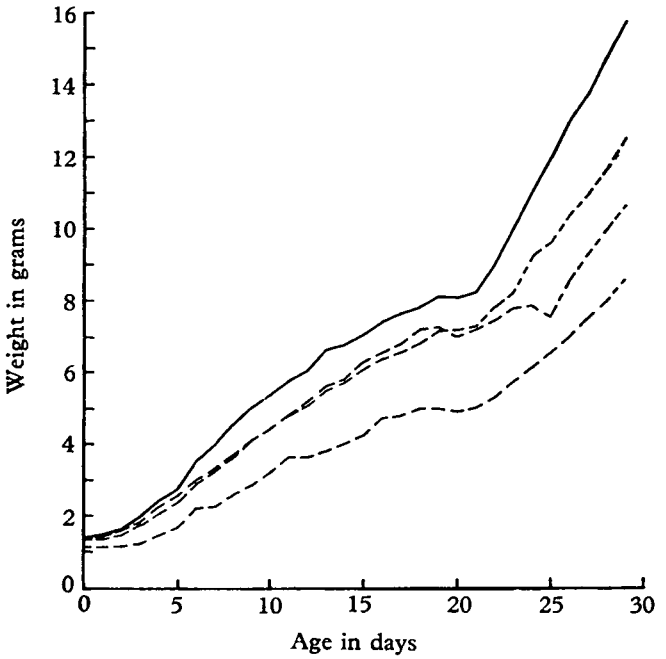

Fig. 1. Average growth curve of 4 normal (solid line) and individual growth curves of 3 anaemic mice (broken lines).

MATERIAL AND METHODS

This report is based on a study of anaemic and normal mice of the following ages: new-born, 7 days, 14 days, 21 days, 28 days, 72 days and 240 days. Each age group was represented by 10 male anaemic mice along with 10 of their normal litter mates (mostly males, except in rare cases where females had to be used). The fact that some of the females might have been heterozygous for the gene *sla* makes no difference to their value as controls, for $+/sla$ females are haematologically normal (see below). The work was confined to male mice as far as possible, because anaemic males ($-/sla$) are easier to obtain than anaemic females (*sla*/*sla*). All mice were descended from a single $-/sla$ male received from Dr Falconer in 1958 which was mated with F_1 females from a cross between the inbred strains CBA/Gr and C57BL/Gr.

Blood was obtained by decapitation. In newborn mice where only a small quantity of blood is available, the same sample of blood was used for the determination of haematocrit and Hb value as advised by Grüneberg (1942*a*). Hb (in g./100 c.c. of blood) was determined by means of an M.R.C. Grey-wedge Photometer calibrated by the oxyhaemoglobin method; the average of triplicate readings was

taken for every mouse. Erythrocytes were counted in a Hawksley improved Neubauer counting chamber. The volume of the packed red blood cells was determined in a Hawksley Micro-Haematocrit centrifuge (5 minutes at 12,000 × gravity). From these three determinations, the mean Hb content of the cells in $\gamma\gamma$, the mean corpuscular volume (M.C.V.) in μ^3 and the mean corpuscular Hb concentration (in %) were calculated in the usual way. Blood films were stained with Leishman's stain, or Giemsa and May-Grünwald stains. Reticulocytes were stained supravitally on slides in a moist chamber with brilliant cresyl blue; 1000 cells were examined in each case. Siderocytes were stained in blood films fixed in methyl alcohol by the Prussian blue technique and counterstained with Biebrich scarlet (for details see Grüneberg, 1942*b*). Red cell diameters were determined by means of a micrometer scale on photographs taken at a magnification × 300 (Attfield, 1951). The fragility of the erythrocytes was measured by a technique described by Wintrobe (1946). Living erythrocytes were studied by means of Ponder's (1929) technique. Sections of liver and spleen (Bouin, H. and E.) and bone marrow (in the tibia; decalcification in a solution containing equal parts of 85% formic acid and 2% sodium citrate; stained with azure II-eosin, as advised by Custer (1932)) were also examined.

RESULTS

Average values of the normal and anaemic red blood pictures are given in Table 2. The rather striking changes in the blood which take place in the mouse between birth and adult life have been described in some detail by Grüneberg (1942*a*). The normal mice in the present investigation agree on the whole rather well with the published data. Differences in the Hb value are largely due to a changed standard (100% on the Haldane scale was formerly equated to 13·8 g. Hb/100 c.c. of blood, but is now considered to be equivalent to 14·8 g. Hb). Other quantitative differences are probably largely due to genetic differences between the stocks and to changes in the management of the colony. The dip in the cell count at 28 days with its associated rise in M.C.V. and M.Hb.C. is probably spurious.

The absolute values of the red blood picture of normals and anaemics are given in Table 2. The behaviour of the anaemia is more easily interpreted from Table 3 where the values of the anaemics are given as fractions of those of their normal litter mates. The anaemia does not change its character appreciably between birth and the age of 72 days. The values for old anaemics (240 days) are much closer to normal. It is uncertain whether this represents a real improvement or whether it is due to the fact that anaemics which deviate much from normality are less likely to survive to that age. Whichever explanation is correct, the younger stages are of more importance for the interpretation of the haematological findings. Taking the average of the relative values for the six younger age groups, the anaemia presents a curiously vague picture. Haemoglobin is down to 68%, the cell count to 76% and the haematocrit value to 72%. Consequently the cells are of nearly normal size (94%) and their Hb concentration is also not much below normal (94%); the mean Hb content of the cells, being the product of the latter two values, is 88%. It appears, then, that the cell count is down to roughly three-quarters of its normal

value, but that the cells produced are only very mildly abnormal as judged by size and Hb concentration. It would thus be an exaggeration to describe the anaemia as either microcytic or as hypochromic though the deviations from normality go in those directions.

Table 2. *Red blood pictures of normal (above) and anaemic mice (below). Average values of* 10 *animals in each group*

Age (days)	Weight (g.)	Hb (g./100 c.c.)	Erythrocytes per cu. mm.	Haematocrit (%)	M.Hb.C. ($\gamma\gamma$)	M.C.V. (μ^3)	M.C.Hb.C. (%)
0–1	1·37	13·7	4,000,000	44·1	34·1	109·7	31·2
7	5·84	10·4	4,600,000	34·9	22·8	76·8	29·7
14	8·58	10·0	5,600,000	34·2	18·1	62·0	29·1
21	11·36	12·4	7,100,000	41·4	17·1	57·9	29·9
28	16·81	12·7	6,500,000	41·0	19·5	62·8	31·0
72	29·01	14·3	8,200,000	46·0	17·6	56·7	31·0
240	32·68	13·1	7,700,000	39·8	17·0	51·6	33·0
0–1	1·29	9·4	3,100,000	33·9	30·6	109·8	27·8
7	4·91	7·0	3,200,000	23·8	20·1	68·5	29·4
14	8·38	7·2	4,400,000	26·9	16·6	61·8	27·0
21	10·23	9·5	5,400,000	32·9	17·3	60·0	28·9
28	14·65	8·1	5,000,000	26·8	16·1	53·0	30·1
72	26·14	8·2	6,200,000	30·4	13·4	49·7	27·1
240	31·05	10·6	6,600,000	32·4	16·1	49·2	32·8

Table 3. *Comparison of normal and anaemic blood pictures (anaemic : normal ratios)*

Age (days)	Mean weight (g.)	Hb (g./100 c.c.)	Erythrocytes (cu. mm.)	Haematocrit (%)	M.Hb.C. ($\gamma\gamma$)	M.C.V. (μ^3)	M.C.Hb.C. (%)
0–1	0·94	0·69	0·78	0·77	0·90	1·00	0·89
7	0·84	0·67	0·70	0·68	0·88	0·89	0·99
14	0·98	0·72	0·79	0·79	0·92	1·00	0·93
21	0·90	0·77	0·76	0·80	1·01	1·04	0·97
28	0·87	0·64	0·77	0·65	0·83	0·84	0·97
72	0·90	0·57	0·76	0·66	0·76	0·88	0·87
240	0·95	0·81	0·86	0·81	0·95	0·95	0·99

In stained blood films, it is apparent that there is no appreciable increase in the number of nucleated red cells at birth. There is a mild poikilocytosis and anisocytosis; the latter finds its expression also in the coefficient of variation for the red cell diameters (Table 4). Red cells of anaemics often look ring-shaped (pessary forms) with a normally stained periphery surrounding an almost unstained centre; normal red cells are usually more uniformly stained.

Red blood cell diameters

Red blood cell diameters were measured at the following ages: new-born, 7 days, 14 days, 21 days and 28 days. Each age group was represented by one normal and

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Table 4. *Distribution of erythrocyte diameters in normal and anaemic mice*

Age (days)	Type	Distribution of erythrocyte diameters (μ) 4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	Total	Mean cell diameter (μ)	C.o.V. %
0–1	Normal	0	0	0	0	0	0	5	17	71	78	40	21	17	1	250	8·744 ± 0·0400	7·225
	Anaemic	1	1	1	2	3	16	43	73	61	27	17	5	0	0	250	7·652 ± 0·0511	10·572
7	Normal	0	0	0	2	6	10	46	85	73	24	3	1	0	0	250	7·584 ± 0·0388	8·094
	Anaemic	0	1	1	8	20	28	49	64	55	22	2	0	0	0	250	7·294 ± 0·0509	11·036
14	Normal	0	0	1	1	11	41	93	63	38	1	1	0	0	0	250	7·152 ± 0·0361	7·997
	Anaemic	1	3	5	7	20	39	75	71	26	3	0	0	0	0	250	6·982 ± 0·0479	10·853
21	Normal	0	1	1	3	41	85	79	34	4	2	0	0	0	0	250	6·726 ± 0·0352	8·430
	Anaemic	2	6	9	37	59	66	50	16	5	0	0	0	0	0	250	6·306 ± 0·0471	11·830
28	Normal	0	3	5	16	67	95	52	11	1	0	0	0	0	0	250	6·402 ± 0·0277	6·859
	Anaemic	0	0	3	30	120	77	18	2	0	0	0	0	0	0	250	6·166 ± 0·0269	6·910

one anaemic mouse from the same litter (for technique see the section on Materials and Methods). The distribution of the diameters, their means and coefficients of variation are given in Table 4. In both normal and anaemic mice (as in man) the erythrocytes are largest at birth and decrease in size with age. But they are always smaller in anaemic animals than in their normal litter mates, the difference rapidly decreasing after the first week. In anaemic mice the coefficient of variation, a measure for anisocytosis, is much higher at first: the fourth week value was, however, almost normal.

Reticulocytes

Reticulocytosis is usually the expression of a rapid output of immature cells occurring as a compensatory measure in an anaemia. The reticulocytic percentage is considerably higher in anaemic mice up to the age of 72 days (Table 5). In older anaemic mice, however, it is almost normal.

Table 5. *The frequency of reticulocytes in normal and anaemic mice*

Number of mice	Age (days)	Normal (%)	Anaemic (%)	Anaemic : normal
10	0–1	83·00	94·23	1·135
10	7	46·74	58·86	1·258
10	14	31·39	49·90	1·589
10	21	13·60	23·49	1·727
10	28	11·21	15·76	1·405
10	72	7·78	10·34	1·329
10	240	4·30	4·96	1·153

Siderocytes

They were counted in five anaemic and five normal mice (from the same litters) at each of the following ages: birth, 7 days and 14 days. Their incidence is given in Table 6. Their number is rather higher in anaemic mice at birth, but the difference is greatly reduced at the age of one week and they disappear in both genotypes at two weeks. As siderocytes disappear from the blood at this stage in normal development, the slightly raised values in the anaemic probably simply reflect a slight general retardation of these animals.

Table 6. *The frequency of siderocytes in normal and anaemic mice*

Age (days)	Normal (%)	Anaemic (%)
0–1	3·70	5·40
7	0·26	0·43
14	0·00	0·00

The erythrocyte fragility test

The test was conducted at the age of 14 and 28 days, two anaemic and two normal mice comprising each age group. In all animals haemolysis started at the concentration of 0·45% NaCl and was complete at about 0·36%. The resistance to hypotonic solutions of NaCl in anaemic mice is thus normal.

Haemopoietic organs

The blood of a new-born mouse is formed by liver, spleen and bone marrow. The liver starts to function on the 12th day of gestation and continues to do so until the animals are about two weeks old. The spleen and the bone marrow begin to function on the 15th and 16th days respectively, and carry on throughout life. All three organs were examined to discover the origin of anaemia. In new-born anaemic mice the liver has considerably fewer haemopoietic foci than in normal mice. This difference is reversed at the age of 7 days, though its magnitude is reduced. At the age of 14 days, the foci have disappeared in both normal and anaemic livers. Whether this longer persistence of haemopoietic foci helps to alleviate the anaemia or merely reflects the general retardation of the anaemic is uncertain. The spleen of normal and anaemic mice at different stages was sectioned. There seems to be no difference between the two genotypes.

For a study of the bone marrow sections of the tibia were cut from mice ranging in age from the new-born to 70 days old. The bone marrow in anaemic mice shows a deficiency of erythropoietic cells at all stages. At birth this deficiency is particularly severe, the bone marrow being composed almost entirely of mesenchymal instead of haemopoietic cells.

Haematology of heterozygous (+/sla) *females*

To find out whether the gene has any effect in the heterozygous state, and whether the use of +/*sla* females as controls was thus legitimate, the haematology of ten such females was studied and compared with that of their normal brothers. There appears to be no difference between the two, and the gene may be regarded as fully recessive as far as haemopoiesis is concerned.

Leucocytes

Examination of leucocytes in stained blood films showed that the leucocytes are apparently unaffected by the gene *sla*.

DISCUSSION

The sex-linked anaemia presents a rather vague haematological picture. The red cell count is reduced to about three-quarters of the normal values, but the cells are only slightly hypochromic, and are little reduced in volume. They are, however, often only faintly stained in the centre as seen in blood films, and their mean diameter is reduced more markedly than their mean volume. This behaviour would seem to indicate that the cells are more pinched-in in the centre but thicker than normal at the rim, with an overall reduction in diameter but scarcely in volume. All this is deduced from the various measurements rather than based on direct observation.

The other haematological findings (such as poikilocytosis, anisocytosis and reticulocytosis) are quite unspecific and common to nearly all anaemias, or, like the slight siderocytosis, can easily be explained by the moderate retardation in development of the anaemics.

Mr B. L. Cohen (Department of Genetics, University of Glasgow) has carried out some additional investigations on which he reports as follows. 'At the request of Prof. H. Grüneberg, I examined the haemoglobin and plasma protein patterns of mice segregating for sex-linked anaemia (*sla*). Blood was taken from a homozygous female (*sla*/*sla*), a heterozygous female (*sla*/+) and a hemizygous male (*sla*/→) of the stock kept by Dr D. S. Falconer at the Institute of Animal Genetics, University of Edinburgh. The plasmas and haemolysates were examined by uni-dimensional starch-gel electrophoresis using the discontinuous buffer system of Poulik (1957). The haemoglobin patterns obtained from all three animals were of the "diffuse" type (Ranney, Smith & Gluecksohn-Waelsch, 1960) and were electrophoretically indistinguishable from the "diffuse" pattern obtained from mice of the inbred strain CBA/Fa. Electrophoresis of the plasma proteins also gave patterns very similar to those obtained from mice of standard inbred strains. There was, however, segregation at the Transferrin locus. Thus both the females examined were apparently heterozygous (phenotype Trf–ab) whilst the male gave the homozygous phenotype Trf–b (for notation see Cohen & Shreffler, 1961).' While this negative result indicates that the Hb of anaemic mice has the same net electrical charge as normal Hb, it does not, of course, prove that it is identical with normal Hb in other respects.

The anaemia appears to be caused by a faulty production of erythrocytes by the liver and the bone marrow in the first few days of life and by the latter alone afterwards. In the case of the liver the fault lies largely in the underproduction of erythrocytes: the anaemic liver has fewer haemopoietic foci at birth than normal, and if their number is higher than normal at 7 days it is presumably because the bone marrow has failed to take over at the normal time and the liver has to go on functioning for a longer period. In the case of the bone marrow, on the other hand, not only are the erythrocytes produced in insufficient number, they may also be abnormal in shape, size and haemoglobin content. The almost total lack of haemopoietic foci in the anaemic bone marrow at birth cannot be the result of delayed growth of anaemic mice alone (the size difference is rather small), but must be more specific. Though the prenatal development of the anaemia has not been investigated, the involvement of the liver leaves no doubt that it must be present in the embryo as well, and may possibly go back to the 13th day of gestation. It remains uncertain whether the yolk-sac is also affected before that, there are very few cells of the primitive generation present at birth.

Genetically, the present anaemia differs from all the others in the mouse in that it is sex-linked. Haematologically, there are also marked differences throughout. The whole series of *W* anaemias (for a recent review see Borghese, 1959) is macrocytic normochromic, and the same applies to the mimic gene of that series, Steel (*Sl*; Bennett, 1956). The flexed-tail anaemia (*f*/*f*; Grüneberg, 1942*a*, 1942*b*) is normocytic hypochromic with many siderocytes. Diminutive (*dm*/*dm*; Stevens & Mackensen, 1958) involves a macrocytic anaemia. The anaemia associated with neonatal jaundice (*ja*/*ja*; Stevens, Mackensen & Bernstein, 1959) is microcytic hypochromic, but strikingly, indeed fatally, so. Hertwig's anaemia (*an*/*an*; Thoms, 1951; Kunze, 1954) is mildly macrocytic and hypochromic. The anaemia associated with

Tail-short (*Ts*/+ ; Deol, 1961) seems to be confined to the primitive generation of red cells derived from the yolk-sac.

While the sex-linked anaemia does not resemble any of the other inherited anaemias in the mouse at all closely, it bears some resemblance to one type of hereditary human anaemia, thalassemia or Cooley's anaemia. Thalassemic homozygotes are clearly anaemic at birth and remain so throughout life. Other points of resemblance between the two anaemias are a proportionately greater reduction in the amount of haemoglobin and haematocrit than in the number of erythrocytes, variability of the size of erythrocytes (anisocytosis), abnormal shape of erythrocytes (poikilocytosis), the confinement of haemoglobin to the periphery of erythrocytes (pessary form) and an increase in the number of reticulocytes. Sex-linked anaemia differs from thalassemia in that it has no effect in the heterozygote (thalassemic heterozygotes are slightly affected) and the fragility of the erythrocytes remains normal (it is often decreased in thalassemia). Also, the bone marrow in sex-linked anaemia is hypoplastic, while it is hyperplastic in thalassemia.

SUMMARY

A sex-linked recessive anaemia (symbol *sla*) in the mouse is responsible for a rather uncharacteristic blood picture which persists virtually unchanged throughout life. The red cell count is reduced to about three-quarters of the normal value, the cells produced differ from normal ones by a slight reduction in the mean corpuscular Hb concentration and a slight reduction in the mean cell volume. As the mean cell diameter is more strongly reduced, it is concluded that the cells must be thicker than normal. There is a reduction of haemopoietic tissue both in the liver and in the bone marrow.

This investigation was suggested to me by Professor H. Grüneberg, F.R.S., to whom I wish to express my thanks for many helpful suggestions and discussions. I am indebted to Dr D. S. Falconer who turned the gene over to this laboratory for haematological study, and to Mr B. L. Cohen for allowing me to refer to some of his unpublished data. I also wish to thank Professor M. Maizels, F.R.S., and Dr G. M. Truslove for help in various ways.

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