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MASTER

Progress Report ~~to accompany~~

~~Seventeenth renewal proposal to the~~

Division of Biology and Medicine
U.S. Atomic Energy Commission
Washington, D.C. 20545

Contract number: AT(11-1)-3264

Title of project: INBORN ANEMIAS IN MICE

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DISCLAIMER

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1. SCIENTIFIC BACKGROUND

The basic purpose of this contract is delineation of inborn anemias of the laboratory mouse, carried out by preparation of genetically homogenous stocks segregating only for anemia-producing genes; by descriptions of each condition at all stages in the life-history; by determination of tissue-sites of primary gene action through transplantation experiments; and by analysis of reactions of normal and anemic mice to a variety of stressful stimuli, including X-irradiation, hypoxia, and phenlyhydrazine poisoning. At present 13 single-gene induced anemias are known in the mouse, plus one with multifactorial inheritance, the autoimmune hemolytic anemia of NZB inbred mice. Effects of anemia-producing mutant alleles at 11 of these loci (an; dm; f; ha; ja; mk; nb; Sl; and Sl^d; sla; sph; and W, W^V, W^J) are currently under investigation at The Jackson Laboratory with support from AEC Contract AT(11-1)-3264. We also have established an NZB/BlN colony susceptible to autoimmune disease.

We plan to analyze all presently known hereditary anemias of the mouse and to apply our findings towards an increased understanding of the genetic control of hemopoiesis, regulation of gene action, mechanisms for erythroid homeostasis, and relations between erythropoiesis and myelopoiesis.

2. TRANSPLANTATION OF MOUSE MARROW CELLS BETWEEN MICE OF DIFFERENT GENOTYPES

A paper entitled: "Chimerism induced by intergenotype transplantation of mouse bone marrow" by S. E. Bernstein, describing investigations carried out with the support of the U.S. Atomic Energy Commission under terms of this contract have just been published in Experimental Hematology (Vol. 22: 69-71, 1972). This paper describes the potentiality of the W/W^V anemic recipient to serve as an assay mouse which may be used to distinguish intrinsic vs. extrinsic defects of erythroid stem cells. It suggests that the W/W^V mouse may also serve (after marrow implantation) as a means of producing, for study, phenotypes which otherwise are neonatally lethal (i.e., ja/ja), and it presents data showing that W/W^V mice were completely converted hematologically to the phenotype of the donor when implanted with marrow from either normal mice (+/+), or mice with Hertwig's anemia (an/an), hemolytic anemia (ha/ha), jaundiced (ja/ja), normoblastosis (nb/nb), or microcytosis (mk/mk). These results suggest that in mice of genotypes an/an, ha/ha, ja/ja, nb/nb, and mk/mk the hematologic defect is intrinsic to their erythropoietic precursor cells. Since reprints of the Experimental Hematology article are not yet available, seven copies of the typed manuscript are included with this progress report.

3. ANALYSIS OF THE HEMOPOEITIC DEFECT OF STEEL ANEMIA MICE EXTRINSIC TO STEM CELLS

In collaboration with Drs. M. S. Altus, A. L. Carsten, and A. C. Upton of SUNY at Stony Brook, we have tested the capacity for hemopoietic colony

formation in spleens of normal and steel anemic mice in situ, and when transplanted into mice of other genotypes. This study resulted in a paper, Altus, M. S., S. E. Bernstein, E. S. Russell, A. L. Carsten, and A. C. Upton, 1971. Defect extrinsic to stem cells in spleens of steel anemic mice. PSEBM 138:985-988.

A summary of this paper follows:

The $\underline{Sl}/\underline{Sl^d}$, +/+, and $\underline{W}/\underline{W^v}$ mouse spleens, residing in situ or transplanted into recipient mice of identical or alternate genotypes, were compared with respect to their abilities to support hemopoietic colony formation by exogenous bone marrow cells inoculated intravenously after whole-body X-irradiation. In contrast to the normal numbers of colonies formed consistently in +/+ and $\underline{W}/\underline{W^v}$ spleens, few or no colonies were formed in $\underline{Sl}/\underline{Sl^d}$ spleens, even when spleens of $\underline{Sl}/\underline{Sl^d}$ and $\underline{W}/\underline{W^v}$ types resided side-by-side in the same abdominal cavity. These findings strengthen the hypothesis that the anemia of the steel ($\underline{Sl}/\underline{Sl^d}$) mouse results from a defect in its hemopoietic tissue matrix rather than in its hemopoietic stem cells of humoral regulatory apparatus.

4. RADIATION SENSITIVITY OF ERYTHROID STEM CELLS AND THEIR ERYTHROID MICROENVIRONMENTS

Several years ago we began a series of experiments designed to yield estimates of the radiosensitivity in vivo of normal and mutant erythroid stem cells, and to provide information on the radiosensitivity of several different erythropoietic inductive microenvironments. This work, differentiating the radiation affects on "seed" (stem cell) from that on "soil" (erythropoietic inductive microenvironment), is still in progress.

Mice of three different genotypes were used in these investigations. They were: +/+ (normal seed, normal soil), $\underline{Sl}/\underline{Sl^d}$ (normal seed, deficient soil), and $\underline{W}/\underline{W^v}$ (deficient seed, normal soil). These mutants, when used as tissue donors and recipients, permit us to make such distinctions with relative ease. Determinations have been made of the dose of the radiation that destroys the curative property of the graftfor half of the recipients (LD_{50}). Thus, after whole-body X-irradiation of the mutants just described, the spleens were removed, grafted to histocompatible nonirradiated anemic recipients, and the improved hematological status of the genetically anemic recipient over a relatively long term (120 to 180 days) was taken as a measure of postirradiation functional condition of the tissues under study. For example, seed deficient, soil normal anemic mice (unirradiated $\underline{W}/\underline{W^v}$) were used as graft recipients (assay mice) to assess the radiation sensitivity of stem cells from +/+ (normal seed, normal soil) when such cells had been irradiated in their indigenous environment.

These investigations were also designed to permit an evaluation of the effect on later potential for therapy of time of residence of the graft in

the irradiated donor. Table A indicates the numbers and kinds of animals employed: the X-ray doses used; the variations in time elapsed between X-irradiation of the donor and implantation of the graft; the data collected to date; and an extremely crude estimate of LDg_{50}. Despite the incompleteness of the data (imposed by a temporary restriction in the number of experimental animals available for these studies which has persisted throughout the current year) several significant trends are apparent. Data from this type of "therapy assay" suggests:

1. Steel stem cells are moderately radiosensitive when irradiated in vivo (with a graft-to-implant interval of one half hour, the LDg_{50} is near 500 R). Studies presented in last year's progress report, in which normal $LD_{50/30}$ values were observed when $\underline{W}/\underline{W^v}$ anemic mice were cured of their anemia by $\underline{Sl}/\underline{Sl^d}$ anemic stem cell implants, gave support to the idea that the radiosensitivity of $\underline{Sl}/\underline{Sl^d}$ stem cells is matrix dependent ("soil" dependent). Hence, the radiosensitivity of the entire animal ($LD_{50/30}$ = 150 R) is not due solely to the inherent radiosensitivity of its erythroid stem cells.

2. Steel splenic tissue progressively declines in its therapeutic effectiveness with increasing time in the irradiated steel donor. This supports the hypothesis that the $\underline{Sl}/\underline{Sl^d}$ environment may be hostile to stem cell replication, a point we will discuss in greater detail in the second unnumbered paragraph.

3. $\underline{W}/\underline{W^v}$ "soil" has normal or possibly even supernormal radioresistance. Accordingly, $\underline{W}/\underline{W^v}$ "soil" is not likely to be responsible for the marked radiosensitivity of the intact $\underline{W}/\underline{W^v}$ mouse ($LD_{50/30}$ = 250-350 R).

4. There is no real decline in the therapeutic potential of either $\underline{W}/\underline{W^v}$ or +/+ "soil," with increasing time, as has been observed with steel "seed."

From the foregoing observations on the modification of therapeutic potential of "soil" with time of residence in the donor, one may state with some certainty that within the time limits specified in these experiments there are no significant migrations of "soil" components into or out of the graft. Alternatively, one could postulate that the lack of change with time implies irreparable initial radiation damage to "soil," which removes any possibility of gross recovery during the 270 day observation period of essential "soil" components, whether they reside in the original donor or in the new recipient.

Thus, though "soil" is only moderately radiosensitive, as determined by the therapy assay (LDg_{50} = 600 R), it represents one of the radiosensitive tissues of significance both to erythroid homeostasis, and to the organism as a whole. The basis of its importance appears to be delayed, or nonexistent, recovery potential. Regrettably we are not yet able to rationalize the discrepancy between our observations and those of Trentin who, using different methods, reported an LD_{50} for splenic hemopoietic inductive microenvironments in excess of 10,000 rad.

In the case of steel "seed", time of residence of the spleen in the irradiated donor does make a difference to its therapeutic potential. Its effectiveness does decline with time. Is this decline due to an out-migration of "seed," or to nonreplacement of splenic "seed" by "seed" emanating from the bone marrow (recruitment)? Does the greater effectiveness of newly transplanted spleens suggest that repair processes proceed more quickly when such cells are transplanted to a more normal environment? Is the steel environment positively or passively hostile to irradiated erythroid stem cells? Frankly, we do not know, but we do know that additional experiments and new types of controls will be required if we are to begin to answer these questions.

As a minimum, it would be desirable to: (1) determine if +/+ "seed" behaves as does $\underline{Sl/Sl^d}$ "seed" under similar experimental circumstances, and (2) confirm the temporal decline in $\underline{Sl/Sl^d}$ stem cells by an independent method, such as the colony-forming-unit assay. The latter method would not assist us in distinguishing between the concepts of: migration, failure of normal recruitment, hostile environment, or quick repair in a normal environment, but would test the validity of the concept of stem-cell decline.

TABLE A

Curative potential of graft irradiated in donor - Time Course Effect

	Spleen donor	non-irrad. recipient	Hours between x-irrad. grafting	Therapeutic success (cured total) of graft X-irradiated at doses indicated*						LDg_{50} est.
				135 R	170 R	210 R	250 R	400 R	650 R	
1.	S1/S1^d	W/W^v	1/2	6/6			6/6	6/8	0/6	>400 R
			3	6/6			3/6			250
	Radiation sensitivity		6	5/5			6/8			>250
	of S1/S1^d stem cells		18	5/5	11/13	1/5	3/8			200
	(seed)		96					0/6	0/6	<400
				300 R	350 R	400 R	600 R	650 R	750 R	
2.	W/W^v	S1/S1^d	1/2			6/8	3/6	6/8	0/6	700 R
			3			7/7	6/8			>600
	Radiation sensitivity		6			6/8	4/6			600
	of W/W^v matrix		18	3/6	5/5	7/9	5/10			600
	(soil)		96					4/6	0/6	600
				200 R	600 R	650 R	750 R	850 R	900 R	
3.	+/+	S1/S1^d	1/2	7/7	0/5	0/6	0/5	0/5		<600 R
			3	6/6	3/6					600
	Radiation sensitivity		6	7/8	2/8					500
	of +/+ matrix		18	7/7	5/7					600
	(soil)		96			0/6	0/7	0/6	0/5	<650

4. Controls:
   (a) Spontaneous cures of untreated anemics: (W/W^v = 0/69) (S1/S1^d = 0/93) during period of experiment.
   (b) Rate of cures in concurrent experiments with non-irradiated spleens transplanted to non-irradiated recipients: W/W^v donor and S1/S1^d recipient = 90% (19/21); S1/S1^d donor and W/W^v recipient = 100% (21/21); +/+ donors and S1/S1^d recipients + 81% (17/21)

* 180 day cures; to be classified as cured hematocrits must exceed values of 45.2 for W/W^v mice and 36.5 for S1/S1^d.

5. PLASMA ERYTHROPOIETIN LEVELS IN STRESSED ANEMIC MICE

In collaboration with Dr. Geoffrey Keighley, Dr. Russell continues studies of erythropoietin levels and responses in W/W^V, Sl/Sl^d, and mk/mk anemic mice and their normal counterparts under ambient atmospheric conditions, during and after prolonged hypoxia, and after whole-body irradiation.

One paper on this work will be published in the near future:

RUSSELL, E. S., and G. KEIGHLEY. The relation between erythropoiesis plasma erythropoietin levels in normal and genetically anemic mice during prolonged hypoxia or after whole-body irradiation. Brit. J. Haematol.

Seven copies of the manuscript were submitted to AEC in 1971.

Recent experiments include successful maintenance of Sl/Sl^d mice in constant hypoxia for more than 20 days without preliminary intermittent exposure. Near the end of this period they finally began to show elevated hematocrit levels. Elevated hematocrits were also seen in microcytic anemic mice exposed to constant hypoxia.

6. METABOLIC CHARACTERISTICS OF MUTANT ERYTHROCYTES

One of our long-term primary research objectives has been to determine the nature of the biochemical lesion encountered in each of the thirteen different hereditary anemias known in the mouse. Such a search has, naturally, led us to look for biochemical derangements in red cell function, specifically for: hemoglobinopathies, nutritional deficiencies, and enzymopathies. However, to date we have discovered neither hemoglobinopathies nor nutritional deficiencies. As indicated in our earlier reports, numerous defects in either the hexosemonophosphate shunt or in the Embden-Meyerhoff pathways are possible, and both have been shown to be causative agents of human hemolytic disease. Recently, therefore, we have devoted our efforts to the enzymology of erythrocytes.

In our continuing efforts with Dr. John Hutton, now of the University of Kentucky, we assayed levels of erythrocyte enzymes involved in the glycolytic pathways and determined quantities of other intracellular substances deemed to be essential to satisfactory erythrocyte function. Our studies utilized mutants with overt hemolytic disease (jaundiced, ja/ja; hemolytic anemia, ha/ha; normoblastosis, nb/nb; microcytosis, mk/mk; and spherocytosis, sph/sph) and the heterozygous carriers of these conditions, which have at least grossly normal hematologic values. Red blood cells from these mutant mice from the genotypes just listed, both homozygous affected and heterozygous carrier individuals, and from highly congenic homozygous normal mice, have been analyzed for the materials listed in Table B. Most of the data have been included in previous progress reports, hence they will not be repeated here. To summarize previous work, in no case have we

seen a significant decrease in the content or activity of any of these substances sufficient to account for the shortened erythrocyte-survival times characteristic of these mutant erythrocytes.

During the past year we have confirmed many of the original observations and added activity values for three enzymes not previously studied. These are: Triosephosphate isomerase, Phosphoglycerate mutase and Enolase (see Table B for a complete list of erythrocyte enzymes and metabolites). Once again, elevated rather than diminished levels of enzymatic activity were encountered in the hemolysates studied, almost certainly reflecting the younger average age of the erythrocytes encountered in these mutants. This position is seemingly strengthened by the existence of a very strong positive correlation between elevated levels of TPI and elevated levels of the other two red cell enzymes PGM and ENOL.

Values for Glutathione, Nicotinamide-Adenine Dinucleotide, and Nicotinamide-Adenine Dinucleotide Phosphate were in the normal range or were higher than normal (Table D), suggesting that the glutathione reductase or methemoglobin reductase systems are nearly normal.

Finding no significant clues in these data as to the causation of any of these blood disorders, we turned our attention to the quantitation of glucose utilization by erythrocytes of normal and anemic mice. In this study, glucose labeled with tritium in position 2 or with ^{14}C in positions 1 or 2 were employed, and the rate of production of tritiated H_2O and ^{14}C-labeled CO_2 were measured, both in the presence of and in the absence of methylene blue, a substance which is known to increase oxygen uptake by red blood cells by mediating oxygen interaction with NADPH.

Preliminary information from these investigations appear in Table E. Here again, there is no direct evidence of glucose dysutilization by any of the mutant red blood cells. In fact only augmented utilization is indicated, leading one to conclude that none of the enzymes involved in the degradation of glucose are grossly defective. Interpretation of the data is a bit complicated, however, since labeled CO_2 could arise in the pentose phosphate pathway, and labeled H_2O could be produced from the interaction of oxydized glutathione and NADPH. Or, as may be the case here, labelled CO_2 or H_2O could result from activity of the citric acid cycle, a cycle which is active in reticulocytes and nucleated red blood cells (which still possess mitochondria), but which is missing from mature mammalian red blood cells. Thus the apparent augmentation of glucose utilization could be attributed to citric acid cycle activity in the immature cells which make up such a large portion of the mixed populations of the blood cells commonly seen in individuals with overt hemolysis.

Though we have labored mightily our results have not provided positive clues as to action of the mutant genes responsible for any of the mouse hemolytic anemias.

TABLE B. ERYTHROCYTE ENZYMES AND METABOLITES

NAME	ABBREVIATION	
ACETYL CHOLINESTERASE	(AChE)	
ADENOSINE TRIPHOSPHATE	(ATP)	
ALDOLASE	(ALD)	
ENOLASE	(ENOL)	--ADDED THIS REPORT
FRUCTOSE 6 PHOSPHOKINASE	(F6PK)	
GLUCOSE 6 PHOSPHATE DEHYDROGENASE	(G6PD)	
GLUTATHIONE	(GSH)	--ADDED THIS REPORT
GLUTATHIONE REDUCTASE	(GR)	
GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE	(GAPDH)	
HEXOKINASE	(HK)	
LACTATE	(LAC)	
LACTIC DEHYDROGENASE	(LDH)	
METHEMOGLOBIN	(MHb)	--ADDED THIS REPORT
NICOTINAMIDE-ADENINE DINUCLEOTIDE	(NADH)	--ADDED THIS REPORT
NICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE	(NADPH)	--ADDED THIS REPORT
PHOSPHOGLUCOSE ISOMERASE	(PGI)	
PHOSPHOGLYCERATE MUTASE	(PGM)	--ADDED THIS REPORT
PYRUVIC KINASE	(PK)	
TRIOSEPHOSPHATE ISOMERASE	(TPI)	--ADDED THIS REPORT
6-PHOSPHOGLUCONATE DEHYDROGENASE	(6-PDGH)	

TABLE C: ENZYMATIC ACTIVITIES IN HEMOLYSATES FROM NORMAL AND ANEMIC MICE[1]

	genotypes of blood donors			
	+/+	ha/ha	nb/nb	mk/mk
1. Values obtained with high levels of substrate.				
ENZYMES				
TPI	350[2]	437	486	1070
PGM	3.8	4.2	7.0	18.0
ENOL	8.1	13	15.9	36.0
2. Values obtained with low levels of substrate.				
ENZYMES				
TPI	46	83	70	169
PGM	--	--	--	--
ENOL	5.7	9.3	12.0	20.3

(1) Preliminary report of work done in collaboration with Dr. John Hutton, Univ. of Kentucky.

(2) Specific Activity (μmol product/min/g Hb at 30 C)

TABLE D. QUANTITY OF GLUTATHIONE (GSH), METHEMOGLOBIN (MHb), NICOTINAMIDE-ADENINE DINUCLEOTIDE (NADH), AND NICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE (NADPH) IN ERYTHROCYTES OF NORMAL AND ANEMIC MICE(1)

SUBSTANCE		Genotypes of blood donors +/+	ha/ha	nb/nb	mk/mk
1. GSH	(μmol/g Hb)	6.4	15.0	10.9	8.1
2. MHB	(%)	0.9	3.9	0.4	1.8
3. NADH	(mμmol/g Hb)	325	296	307	654
4. NADPH	(mμmol/g Hb)	39	54	57	66

(1) Preliminary report of work performed in collaboration with Dr. John Hutton, Univ. of Kentucky.

TABLE E. QUANTITATIVE GLUCOSE UTILIZATION BY ERYTHROCYTES OF NORMAL AND ANEMIC MICE.[1]

	genotypes of blood donors			
	normal control (+/+)	ha/ha	nb/nb	mk/mk
1. Tritiated H_2O formed from				
glucose-2-^3H w/o MB[2]	6.8[3]	15.7	16.5	45.9
MB	11.0	34.4	29.2	90.6
2. $^{14}CO_2$ evolved from				
glucose-1-^{14}C w/o MB	1.02	2.32	1.57	2.54
MB	26.7	78.6	84.2	164.8
3. $^{14}CO_2$ evolved from				
glucose-2-^{14}C w/o MB	0.05	1.03	1.10	1.02
w MB	7.6	7.4	10.6	32.9
4. ratio $^{14}{}_1CO_2/^3HHO$ w/o MB	0.15	0.15	0.10	0.06
w MB	0.28	0.09	0.13	0.20
5. ratio $^{14}{}_2CO_2/^{14}{}_1CO_2$ w/o MB	0.04	0.44	0.77	0.40
w MB	0.28	0.09	0.13	0.20

(1) Preliminary report of work done in collaboration with Dr. John Hutton, Univ. of Kentucky.

(2) w/o MB = without methylene blue; w MB = with methylene blue.

(3) values in μmol/g Hb/hr.

7. PYRROLE PIGMENTS IN NORMAL AND CONGENITALLY ANEMIC MICE

The levels of free erythrocyte protoporphrin, serum bilinogen, and fecal urobilinogen each provide information on hemoglobin metabolism and its abnormalities, and are useful in elucidating the mechanisms of anemia. In collaboration with Drs. Martha Kreimer-Birnbaum and Robin Bannerman of SUNY at Buffalo, we have carried out systematic observations in adult and fetal normal mice of several inbred strains and in mice with hereditary anemia. This investigation has resulted in a paper:

KREIMER-BIRNBAUM, M., R. M. BANNERMAN, E. S. RUSSELL, and S. E. BERNSTEIN. Pyrrole pigments in normal and congenitally anemic mice. Comp. Biochem. Physiol., In press.

A summary follows:

1. Values for fecal urobilinogen, serum bilirubin, and free erythrocyte protoporphyrin are reported for adult hematologically normal mice from six genetically homogenous stocks.

2. The same values were determined for anemic mice with six different kinds of hereditary anemia, each differing from its normal counterpart essentially only by the anemia-producing mutant allele.

3. Mice with two kinds of severe hemolytic anemia (nb/nb and ha/ha) showed elevated serum bilirubin and greatly increased fecal urobilinogen.

4. Mice with microcytic hypochromic anemia (mk/mk), which may be due to an unusual anomaly of iron metabolism, and (sla/Y) mice with sex-linked hypochromic anemia involving defective iron transport, both showed increased free erythrocyte protoporphyrin and moderate increases in fecal urobilinogen, suggesting an element of increased hemoglobin catabolism combined with defective hemoglobin synthesis.

5. Free erythrocyte protoporphyrin determinations were also carried out for normal 15-day fetuses. Levels were extremely high in normal fetal red cells, probably associated with active hemoglobin synthesis in this young, rapidly expanding cell population.

6. Free erythrocyte protoporphyrin levels in 15-day flexed-anemic fetuses were lower than those in normal fetuses, but markedly above adult levels. The siderocytic anemia of flexed fetuses can not be attributed to absolute lack of free erythrocyte protoporphyrin.

Seven typed copies of this manuscript are enclosed with this progress report.

8. IRON DISTRIBUTION AND METABOLISM IN MICE WITH HEREDITARY ANEMIA

Microcytic (mk/mk) mice appear to handle iron differently than do normal mice, but their problem is not restricted to defective intestinal

iron-transport, as is that in mice with sex-linked anemia. Since Drs. Robin Bannerman and John Edwards of SUNY at Buffalo are particularly interested in hypochromic anemias and iron utilization, Dr. Russell has collaborated with them in a study of iron utilization in microcytic mice, which has resulted in a publication:

BANNERMAN, R. M., J. A. EDWARDS, M. KREIMER-BIRNBAUM, E. C. McFARLAND, and E. S. RUSSELL. Hereditary microcytic anemia in the mouse; studies in iron distribution and metabolism. British. J. Haemat., In press.

A summary follows:

"Iron distribution and metabolism have been studied in hereditary microcytic anemia of the mouse (gene symbol mk), an autosomal recessive trait characterized by hypochromia and microcytosis. Evidence of iron deficiency was found in the form of depleted body stores, hyposideraemia, an increased total iron binding capacity of the plasma and a high free erythrocyte protoporphyrin level. However, the failure to find either rapid clearance and high utilization of tracer doses of ^{59}Fe or a complete response to parenteral iron treatment indicated that simple iron deficiency was not the cause of the anemia. It is suggested that a generalized impairment in the cellular uptake of iron involving the transfer of iron from the intestinal lumen to the mucosa and from the plasma to the erythroblast may provide a unitary explanation of hereditary microcytic anemia."

Seven copies of this manuscript are enclosed with this progress report.

9. ESTABLISHMENT AND STUDY OF SEX-LINKED ANEMIA

Male mice hemizygous (sla/Y) and female mice homozygous (sla/sla) for sex-linked anemia have a hypochromic anemia which has been shown to result from deficiency of iron supply to the hemopoietic system, at least after birth. The cause of the iron deficiency is a defect in iron transport across the intestinal epithelium. Because we wished to study this anemia, to build up highly congenic mouse stocks with and without the sla gene, and to provide a source for such animals for many interested investigators, we imported the sla gene into The Jackson Laboratory in 1969. Since then have systematically crossed sla/Y males to C57BL/6J females to establish a congenic stock. After some initial difficulties, this breeding program is now proceeding well. Recently we have started a stock with combined sex-linked anemia (sla) and tortoise (To) in repulsion (+ sla/To +). Both loci are on the X-chromosome, rather closely linked. We plan to use this linkage as a device to distinguish + sla/+ (solid color) carrier females from noncarrier To +/+ + (mottled) females in segregating populations. This eliminates many time consuming hematologic tests and progeny tests. We also hope, by keeping careful records in maintaining this stock, to find sla To/+ + recombinants, and to establish map distance between these two genetic loci.

We have characterized the sla/Y and sla/sla anemias at birth, 3 weeks, and 5 weeks, and have made preliminary observations of anemia in sla/sla and sla/Y fetuses.

10. PREPARATION OF IMPROVED STOCK CARRYING HERTWIG'S ANEMIA

Hertwig's anemia (an/an) is an extremely interesting condition for two reasons:

1. Although it is a macrocytic anemia, similar in many ways to W/W^v and Sl/Sl^d, affected anemic animals are normal in color, with no white spotting, and their gonads appear at least close to normal.

2. Anemic an/an mice are really pancytopenic, with reduced numbers of leukocytes and platelets, and the study of these associated defects is extremely important both for understanding an gene action and for assaying usefulness of this mutant as a model of human pancytopenia. This study has been progressing slowly, however, due at least in part to an insufficient supply of an/an mice. All studies of mouse gene-action depend to a considerable degree on transplant of tissues between individuals of differently affected genotypes. Until recently this technique had only very limited usefulness for an/an analysis, since the genetic background of mice carrying an was different from that of W/W^v (see Paragraph 2 in this progress report). We have now transferred a segment of chromosome 4 (Linkage Group VIII), carrying B^{lt} an, to the WB/Re inbred strain.

This stock has now reached N10, so that, except for that short region of chromosome 4 (Linkage Group VIII), 99% of the genome of WB/Re - B^{lt} an/+ +, N10, is one of the WB/Re genotype. These heterozygotes can be crossed to C57BL/6--B^{lt} an/+ + mice to provide viable, reasonably vigorous $WBB6F_1$-B^{lt} an/B^{lt} an anemic mice and highly congenic normal littermates. A colony of 20 matings has already been set up to produce $WBB6F_1$-B^{lt} an/B^{lt} an mice, and the size of this breeding colony will be increased as more parental stock animals become available. This new, viable stock of Hertwig's anemic mice should allow us to undertake many experiments which have up to now been postponed due to lack of suitable animal material. Among our first studies will be analysis of the leukopenia characteristics of an/an mice.

11. ESTABLISHMENT AND CHARACTERIZATION OF NZB/BlJ MICE AT THE JACKSON LABORATORY

NZB/BlJ mice develop a very interesting autoimmune hemolytic anemia which has attracted considerable attention among immunologists. We imported the inbred strain in 1969-1970 to study this anemia and to assure interested investigators of a continuing source of NZB mice. The stock is now well established, and we have determined blood counts, hematocrit levels, reticulocyte percentages, and hemoglobin content at ages from 1 month to over one year, in both sexes. Further analysis is planned.