95.1 percent of glucose. When the alkali-insoluble fraction from seed was treated with chlorite and then reextracted with a mixture of 24 percent KOH and 4 percent borate, a further quantity of almost pure mannan was extracted, and the glucose content of the residue increased by 7 to 8 percent. The seeds contain no lignin, and this loss of mannan probably represents either degradation or removal of masking protein, thus allowing further hemicellulose to be extracted. Nitrogen determinations indicated that chlorite treatment reduced the protein content of the fraction from about 1.6 percent to less than half of this.

Hemicelluloses have been extracted from plant cell walls with various concentrations of alkali up to 24 percent KOH. Extraction with 24 percent KOH leaves as a residue the so-called "α-cellulose" which, though rarely, if ever, homogeneous, usually consists very largely of glucose units. Jones *et al.* (*9*) found that 24 percent KOH containing 4 percent boric acid was most effective in extracting mannose-containing hemicelluloses from woody tissues, although Hamilton and Quimby (*10*) and Rapson and Morbey (*11*) claim that KOH is less effective than NaOH in extracting mannans. However NaOH had little effect on *Melampyrum* seed cell wall extracted with KOH/borate as in Table 1. The procedures recommended (10 and 15 percent NaOH at 25°C; *10, 11*) produced little loss of weight and no change in composition.

In algal cell walls the alkali-insoluble fraction has frequently been found to contain considerable amounts of mannan. Thus the cell wall of *Hydrodictyon africanum* after extraction with a mixture of 24 percent KOH and 4 percent borate contained equal parts of mannose and glucose residues (*12*). There was appreciable mannan left after the cell wall of *Porphyra umbilicalis* was extracted with 20 percent NaOH (*13*), and an alkali-insoluble fraction was obtained (*14*) from *Porphyra* sp., which appeared to contain mannan only. In contrast, cell walls of higher plants apparently only rarely contain large amounts of mannan. Several members of the Palmaceae (the best known being the ivory nut *Phytelephas macrocarpa*) produce very hard seed endosperms which contain considerable mannan. However, as the mannan of the ivory nut has been variously stated to be partially (*15*) or completely (*16*) alkali-soluble, it is not known how closely these mannans resemble that of *Melampyrum*.

The role of the mannose residues in the *Melampyrum* seed cell wall is not known, nor is it known whether the mannose units occur with glucose as glucomannan or form separate mannan polymers, or whether the material is microfibrillar. In the endosperm of the ivory nut, part of the mannan found (*15*) appeared to be microfibrillar, although x-ray data indicated an amorphous, and not a crystalline, structure. It may be that *Melampyrum* and ivory nut are both examples of a reserve mannan laid down around the more structural glucan (cellulose). That at least part of the mannan material in *Melampyrum* seed is laid down during later development is indicated by the larger proportion of mannose units in ripe seed than in unripe seed, and by the fact that chlorite treatment liberates part of the mannan but no glucan. Seed dormancy in *Melampyrum* (*3*) is associated with inability of the embryo to hydrolyze the endosperm cell walls. Excised dormant embryos grow well on aqueous endosperm extracts, which contain large amounts of sucrose. However, this sucrose is available to the embryo in vivo only after breakdown of the endosperm cell walls.

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Sex-Linked Anemia:
A Hypochromic Anemia of Mice

Abstract. *This hereditary anemia is most severe in young mice and tends to diminish with increasing age. Erythrocytes show great variation in size and form, with hypochromia and formation of target cells. Though the anemia occurs on a normal diet, it responds rapidly to iron-dextran injection. It may represent an unusual primary disturbance of iron metabolism.*

Sex-linked anemia in mice was discovered in 1958 by Falconer and Isaacson, who showed that the mutant gene *sla* is carried on the X chromosome and manifested as a recessive (*1*). Grewal reported further on the genetics and on some features of the anemia (*2*). In hemizygous males and homozygous females he noted that red cell counts (and hemoglobin) were reduced to approximately 75 percent of the normal values, with slight reduction in mean corpuscular hemoglobin and mean cell volume. No change with age was reported.

Our present studies, undertaken to clarify the mechanism of this anemia, were made on mice inbred from a small stock of mixed ancestry obtained from Falconer. Morphologically, blood smears in young anemic mice show marked variation in red cell size and shape with many hypochromic and microcytic cells, some cellular fragments, other cells which are macrocytic with marked polychromasia, and an increased number of large target cells (Fig. 1). The appearance somewhat resembles that of the thalassemias in man (*2*) and, less closely, that of very severe iron-deficiency anemia and other rarer hypochromic anemias, including some types of pyridoxine-responsive anemia.

The anemia can easily be detected soon after birth in the affected mice, and it is initially severe with hemoglobin levels of 4 to 8 g/100 ml at age 30 to 40 days. There is somewhat less reduction in hematocrit, so that the mean corpuscular hemoglobin concentrations are very low (19 to 25 percent). There is moderate reduction in red cell count (3 to 6 $\times$ 10^6 cells per cubic millimeter), with normal or slightly reduced mean cell volume.

Despite considerable individual variation, the anemic mice show a constant tendency to correct the anemia with increasing age. There have been a very

few exceptions among young mice which died, apparently of anemia, with hemoglobin levels of less than 4 g/100 ml, at under 50 days of age. Otherwise, both individually and collectively,

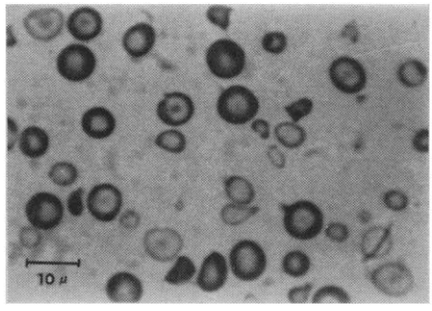

Fig. 1. Blood smear of a young, affected, male *sla* mouse, showing variable red cell morphology, with anisocytosis, poikilocytosis, polychromasia, cell fragments, and target cell formation.

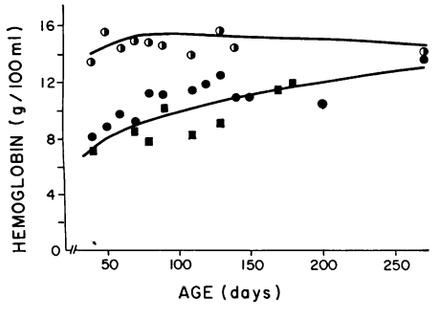

Fig. 2. Hemoglobin concentrations in hemizygous *sla* males (■), homozygous *sla* females (●), and heterozygous *sla* female carriers (◑), at different ages. Each point is the mean value from groups of 6 to 12 animals observed serially.

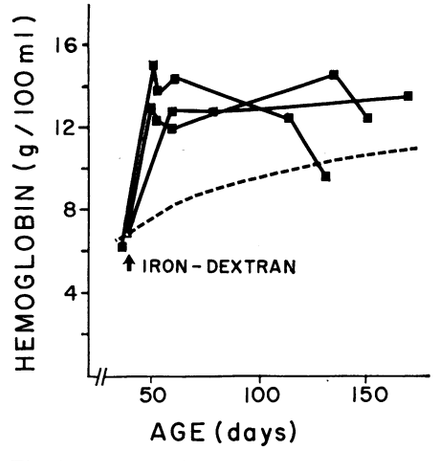

Fig. 3. Hemoglobin concentrations in three anemic *sla* mice after single intraperitoneal injections of iron-dextran (0.01 ml; 1 mg Fe; diluted with saline immediately before injection), compared with the expected curve from Fig. 2 (broken line).

hemoglobin levels rise towards normal values (Fig. 2), and the morphologic picture tends to become more nearly normal. These findings are in contrast to Grewal's original observations (*2*). The anemia occurs and tends to correct itself with time when the mice are on a standard laboratory diet containing apparently adequate amounts of iron, pyridoxine, and other substances (see *3*).

Heterozygous female carriers do not show any significant anemia (Fig. 2), but minor morphologic changes in blood smears can often be detected.

The striking morphologic appearances in the anemic mice prompted us to try treatments with various substances. Anemic *sla* mice were treated with intraperitoneal injections of androgens [which may stimulate release of endogenous erythropoietin (*4*)], pyridoxine [since human pyridoxine-responsive anemia is characterized by a similar morphologic picture (*5*)], iron-dextran, dextran alone, and saline alone. The only treatment to produce an effect was iron-dextran, after which there was an unmistakable and sustained rise in hemoglobin levels toward normal values (Fig. 3).

Preliminary experiments with Fe^{59} indicate that the absorption of inorganic iron is in the same range as in normal, nonanemic mice. The utilization of injected tracer doses of Fe^{59} for hemoglobin formation is also within the normal range in preliminary studies. Determinations of total body iron show that it is not reduced in anemic mice. The transferrin pattern observed by starch-gel electrophoresis is the same as that of normal mice, as reported previously (*6*), and we have been able to demonstrate by autoradiography and by direct counting that it binds Fe^{59}.

Despite a tendency to correction with time, and apparent amelioration by intraperitoneal administration of relatively massive amounts of iron as iron-dextran, this anemia appears not to be due to a simple deficiency of iron nor to defective iron absorption. It is likely that the mechanism may be a more unusual primary defect in iron metabolism.

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Simian Virus 40: Replication in the Presence of Specific Antiserum and Adenovirus 4

Abstract. *Twelve consecutive passages of simian virus 40, made in the presence of adenovirus 4 and antiserum to simian virus 40, indicated that simian virus 40 would multiply almost indefinitely under these conditions. The adenovirus and the simian virus had previously replicated together in the absence of antiserum. These data support the conclusion that the SV-40 genome is incorporated within the protein coats of adenovirus 4.*

We have reported the bizarre phenotypic properties of simian virus 40 (SV-40) which had originally contaminated a strain of adenovirus 4 and which subsequently persisted after six cell-culture passages in the presence of antiserum to SV-40. This contaminant, SV-40, possessed the antigenic and thermal stability properties of the adeno-

Table 1. Titers of SV-40 and adenovirus 4 during seven consecutive passages in tissue culture. The titers of SV-40 are given as $\log_{10}$ $TCID_{50}$ per 0.2 ml of culture supernatant, titrated in cercopithecus kidney cell monolayers. The titers of adenovirus 4 are given as $\log_{10}$ $TCID_{50}$ per 0.2 ml culture supernatant, titrated in human-embryo kidney cell monolayers.

Passage No.	Cell type	Titer		Cumulative dilution ($\log_{10}$)	Passage duration (days)
		SV-40	Adenovirus 4		
6	CMK*	3.1		0	
7	HEK†	3.0		−1.3	5
8	HEK	4.0	5.5	−2.6	3
9	HEK	3.3	5.8	−4.3	2
10	HEK	3.5	4.5	−6.0	2
11	HEK	2.5	5.0	−7.7	3
12	CMK	3.0	5.5	−9.4	5

* Cercopithecus kidney cell monolayers. † Human-embryo kidney cell monolayers.