male. The "syndrome" observed in *S. beldingi* (providing that the X chromosome is aberrant and we think it is) resembles that in fertile XO mice (*5*) rather than X-chromosome deletion in humans, where there is considerable abnormality of primary or secondary sexual characteristics or of both (*6*).

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11 October 1965

Cell Wall of Melampyrum lineare Seed: Carbohydrate Components

Abstract. *Extraction with alkali of cell wall material from the seed of* Melampyrum lineare *leaves a residue (62.7 percent) which on hydrolysis yields over 70 percent mannose. Hydrolysis of the alkali-soluble hemicellulose fraction of the cell wall also yields largely mannose. In contrast, cell wall isolated from* Melampyrum *stem contains over 95 percent of glucose residues in the alkali-insoluble fraction, and mostly xylose residues in the hemicellulose.*

The cell walls of higher plants contain various sugar residues in addition to that of D-glucose (*1*). However, most of the material containing units other than glucose can normally be extracted as "hemicellulose" with alkaline solvents. The residue, the so-called "α-cellulose," normally consists almost entirely of glucans, particularly cellulose. We report here studies of the *Melampyrum lineare* seed, whose cell wall yields an alkali-insoluble fraction consisting largely of mannose units.

Melampyrum lineare Desr. (*2*), a member of the Scrophulariaceae, is a green root parasite indigenous to the northern forests of North America. After forming root connections to a host plant, *Melampyrum* produces copious flowers and fruit, each capsule typically producing four ellipsoid seeds. The ripe seed is 1 to 3 mm in length and contains at one end an elongated embryo embedded in endosperm which occupies the remainder of the seed space. The embryo is dormant until activated (*3*), when it grows at the expense of the endosperm. The nonactivated embryo occupies up to 40 percent of the length of the seed and 10 percent of the volume, and it represents 1.5 percent of the dry weight. The soft, fibrous endosperm tissue of the ripe seed is composed of polygonal cells (average inner diameter, 61 μ) with thickened walls (average diameter, lumen to lumen, 11.2 μ). The cell wall is composed of a middle (nonpitted) lamella (about 2.8-μ thick) surrounded by inner (pitted) lamellae bordering the cell lumina. The inner lamella stained with a solution of iodine in potassium iodide followed by sulfuric acid, and also with zinc chloride iodide (*4*), but the middle lamella did not react. No part of the seed stained with phloroglucinol–hydrochloric acid (*4*), an indication of the absence of lignin.

Dormant whole seeds were cleaned and milled; they were then subjected to successive exhaustive extraction with benzene, 80 percent ethanol, and hot water. These extractions removed respectively 15.8, 22.5, and 7.7 percent of the original dry weight, and left a residue (54.0 percent) of cell-wall holocellulose. The holocellulose was then extracted with alkali (Table 1) to remove hemicellulose components. A similar extraction was carried out on unripe seeds (Table 1). These seeds were obtained from immature seed capsules at which time the seed was soft and pulpy, although the endosperm cell walls were of approximately the same thickness as those in the ripe seed. Stem holocellulose prepared similarly from fresh *Melampyrum* stems was first delignified by chlorite treatment (*5*) and then extracted with alkali.

Samples of the various holocellulose components were then hydrolyzed by digestion with 72 percent sulfuric acid at 24°C; the digest was diluted to 2*N* and autoclaved for 2 hours at 15 lb (2 atm, absolute). After neutralization, sugars were separated on paper chromatograms with a solvent mixture of ethyl acetate, pyridine, and water (8:2:1) and one of ethyl acetate, acetic acid, and water (9:2:2); they were located by spraying with *p*-anisidine hydrochloride (*6*) or aniline hydrogen phthalate (*7*). Hemicellulose hydrolyzates had the following approximate compositions. Potassium hydroxide (5 percent) extract from seed (ripe and unripe) yielded mannose, glucose, galactose, and arabinose in approximate proportions of 3:1:1:0.5, respectively. Potassium hydroxide (24 percent) plus borate (4 percent) extract from seed yielded mannose only. Potassium hydroxide (5 percent) extract from stem yielded xylose, galactose, arabinose, and rhamnose in approximate proportions of 12:1:1:0.5. Potassium hydroxide (24 percent) plus borate (4 percent) extract from stem yielded xylose, galactose, glucose, mannose, arabinose, and rhamnose (1:1:1:1:0.5:0.2).

Table 1. Fractionation of holocellulose obtained from *Melampyrum* seed and stem. Each alkali extract was the result of three successive 12-hour treatments at 24°C, each with fresh solvent.

Fraction	Weight loss (%)		
	Ripe seed	Unripe seed	Stem
Chlorite delignification			36.2
5% KOH	26.5	30.9	30.1
24% KOH + 4% borate	10.8	9.7	10.3
Alkali insol. residue	62.7	59.4	23.4

The composition of hydrolyzates from alkali-insoluble residue was determined by the method of Wilson (*7*). Results are shown in Table 2. Mannose from seed cell wall (alkali-insoluble material) was identified as the phenylhydrazone (*8*), mp 194° to 196°C; the melting point was not depressed by admixture with authentic D-mannose phenylhydrazone. Infrared spectra of the two samples were identical.

In the alkali-insoluble fraction from seed cell wall, mannose accounted for 74.4 percent (ripe seed) and 73.2 percent (unripe seed) of the sugar residues. In contrast the corresponding fraction from stem cell wall yielded

Table 2. Composition (percent) of alkali-insoluble residues from *Melampyrum* cell wall.

Sugar	Seed		Stem
	Ripe	Unripe	
Galactose	4.2	2.7	0.8
Glucose	19.0	21.9	95.1
Mannose	74.4	73.2	2.2
Arabinose	2.4	2.2	0.7
Xylose			.9
Rhamnose			.3

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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14 December 1965

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