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8. Daily smears were taken from females to determine their cycling pattern. In most instances two cycles were followed before we used an animal in the study.
9. Recording electrodes for extracellular population responses were glass micropipettes (1 to 5 μm tip diameter filled with 2*M* NaCl). Stimulation was by concentric, bipolar stainless steel electrodes placed in the afferent fiber path (Schaffer collateral system). The stimulation-evoked, monosynaptic population potentials were displayed on an oscilloscope and recorded for later analysis.
10. Field potentials were elicited by stimulation at 0.2 per second with intensities from below threshold to response maxima. Stimulus duration was 0.1 msec. Shown are data from stimulation intensities chosen to elicit approximately a 1-mV population spike in the control period before steroid treatment. We used the same stimulus protocols before and after steroid administration.
11. The normal incubation medium (Earle's solution) in the slice chamber was replaced with medium containing steroid by means of a push-pull syringe system. Undisturbed electrophysiological recordings can be maintained during and after the 5-minute period required for medium exchange. The dose was chosen to approximate physiological concentrations of steroid.
12. Medium exchange in the controls yielded either no change or a transient increase (10 to 12 percent) in population spike amplitude that recovered to preexchange values within 10 minutes. The transient enhancement of excitability in control experiments may be attributed to the effect of perfusion with fresh medium or the elimination of metabolic products.
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Ferritin Synthesis by Human T Lymphocytes

Abstract. *Mononuclear cells from peripheral blood of normal humans, unselected spleen cells from patients with Hodgkin's disease, and selected T and non-T lymphoid cells from normal peripheral blood and from the spleens of Hodgkin's disease patients were examined for de novo synthesis and secretion of ferritin. After precipitation of labeled lysates and supernatants from unseparated and selected T cells with antiserum to human liver ferritin, two bands were visible on sodium dodecyl sulfate–polyacrylimide gel analysis. The two bands were detected in molecular weight regions 19,000 and 21,000, which are thought to represent the L and H subunits of the ferritin molecule, respectively. The slower band (subunit H) was more radioactive than the faster band (subunit L). The H subunit is found in greater amounts in the serum of some tumor patients, but its cellular origin has not been established. The present findings indicate that cells of the immune system contribute to the synthesis and secretion of a ferritin molecule with a high proportion of H subunits.*

It has been suggested that cells of the immune system participate in the recognition and binding of iron as part of their postulated role in surveillance (*1*, *2*). We have examined several aspects of the interaction of iron and iron-binding proteins with lymphoid cells (*3*-*6*) and macrophages (*7*, *8*). In a recent immunofluorescence study of the distribution of transferrin, ferritin, and lactoferrin in selected populations of human peripheral blood lymphoid cells, we observed an association of transferrin and ferritin with T lymphocytes (erythrocyte rosette-forming cells) but not with B lymphocytes (surface immunoglobulin-bearing) (*9*). The presence of intracytoplasmic transferrin and ferritin in T lymphocytes could constitute a basis for the control exerted by T lymphocytes on a number of other biological systems known to require iron for their normal function, such as erythropoiesis (*10*), cell division (*11*), collagen synthesis (*12*), and bacterial and tumor cell growth (*13*-*16*). However, detection of proteins in cells by immunofluorescence does not discriminate between uptake and de novo synthesis of these products by the cells. By using a sensitive immunoprecipitation assay developed to measure the synthesis of murine terminal deoxynucleotidyltransferase (*17*), we demonstrated that human T lymphocytes (*18*) synthesize and secrete ferritin.

Table 1. Summary of experimental data.

Source of cells (*18*)	Experiment	Cell fraction	ERFC* (%)
Peripheral blood	1	PBM	
		T	99
	2	T	96
	3	T	98
	4	T	97
	6	T	94
Spleen	7	Unseparated	
		T	
		Non-T	
	8	Unseparated	
		T	
		Non-T	
	9	Unseparated	36
		T	82
		Non-T	8
	10	Unseparated	33
		T	87
		Non-T	3

*Sheep erythrocyte rosette-forming cells.

Table 1 shows the cell populations analyzed. Synthesis and secretion of ferritin were observed in unseparated peripheral blood mononuclear (PBM) cells and spleen cells and in T lymphocytes separated from both sources (Fig. 1). After precipitation of labeled cell lysates and supernatants with antiserum to human liver ferritin and reduction of the resultant precipitates, two bands were visible in sodium dodecyl sulfate–polyacrylamide gel (SDS-PAGE). The two bands migrated to the molecular weight regions to which subunits of the human ferritin molecule were previously found to migrate (*19*, *20*). There is considerable evidence that these bands represent de novo synthesis of ferritin. First, purified, unlabeled ferritin isolated from human spleen and heart (*21*, *22*) and electrophoresed under the same conditions showed two bands in the same molecular weight regions (Fig. 1, J and K). Second, identical bands were observed with purified iodinated ferritin isolated from spleen of Hodgkin's disease patients (Fig. 1, H and I). Third, precipitation of T cell lysates with antiserum previously absorbed with purified human spleen ferritin resulted in disappearance of the bands (Fig. 1, F and G). Finally, these bands were not detectable in the same material after precipitation of control samples with normal rabbit serum (Fig. 1A).

In all metabolically labeled preparations of unseparated PBM and purified T cells, the slower band (the H subunit) was more radioactive than the faster band (the L subunit), as illustrated in the representative sample (Fig. 1, B and C). A similar pattern is also seen with Coo-

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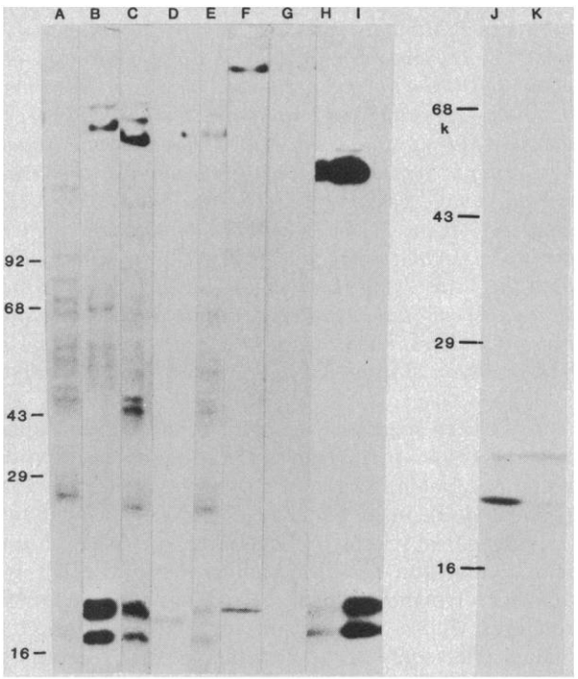

Fig. 1. (A to E) Sodium dodecyl sulfate–polyacrylamide gel analysis of [^{3}H]leucine-labeled cell extracts and supernatants from fractionated spleen cells from Hodgkin's disease patients (*18*). (A) T cell lysate precipitated with normal rabbit serum (7.8×10^5 count/min). (B) Identical lysate precipitated with antiserum to ferritin (ASF). (C) T cell supernatant precipitated with AFS (10^6 count/min). (D) B cell lysate precipitated with ASF (7.8×10^5 count/min). (E) B cell supernatant precipitated with ASF (10^6 count/min). (F and G) Labeled cell lysate from fractionated PBM. (F) T cell lysate precipitated with ASF. (G) Identical sample precipitated with ASF previously absorbed with human spleen ferritin. (H) Iodinated human spleen ferritin, unreduced sample (no 2-mercaptoethanol in sample buffer). (I) Iodinated human spleen ferritin, reduced sample. (J) Coomassie blue-stained proteins in purified human spleen ferritin and (K) in human heart ferritin.

massie blue staining of human heart ferritin (Fig. 1K) (*19*).

Since the metabolic labeling of cells synthesizing ferritin was done with [^{3}H]leucine, the greater intensity of the H band might merely reflect its higher leucine content. However, amino acid analysis of the H and L subunits indicates the same leucine content for both; that is, 20 and 19 residues per subunit, respectively (*19*). This and the staining results suggest greater synthesis of the H than the L subunit.

It has been reported that the serum ferritin of tumor patients contains a higher proportion of H subunits (*23, 24*). The significance of this is unclear because it is not known which cell types contribute to synthesis of the H subunit. The present findings suggest that lymphoid cells themselves participate in the synthesis of this ferritin in normal and Hodgkin's disease patients.

Evidence for the synthesis of ferritin by lymphocytes and PBM cells was presented previously (*25, 26*). We have now demonstrated ferritin synthesis by a selected lymphocyte population. Furthermore, our results indicate that T lymphocytes synthesize ferritin in greater amounts than non-T lymphoid cells (Fig. 1, D and E). Ferritin has been detected on the surface of T lymphocytes from Hodgkin's disease patients (*27*), and it is clear from the present results that more ferritin-associated radioactivity was precipitated from the T cell extracts than from identically labeled non-T extracts. Also, since the extracts are first routinely cleared with *Staphylococcus aureus*, which precipitates a significant part of the material synthesized by B cells (immunoglobulin), ferritin may be synthesized at a much higher rate by T cells than by B cells. Further cases must be examined to confirm this.

The finding of ferritin synthesis and secretion by fractionated T lymphocytes is compatible with the hypothesis that surveillance is mediated at least in part by iron-binding proteins (*1, 2*), and may offer a biochemical basis for some of the roles exercised by T cells in the control of other biological systems.

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18. Heparinized venous blood was obtained from healthy volunteers. Spleens were removed from untreated patients with Hodgkin's disease at staging laparotomy. Peripheral blood mononuclear cells were isolated from venous blood by the method of A. Böyum [*Scand. J. Clin. Lab. Invest. Suppl.* **97**, 77 (1968)]. Spleen cell suspensions were obtained after mincing the spleen tissue in Hanks balanced salt solution (HBSS) (Gibco) and separating the resulting suspension with Ficoll-Hypaque (Lymphoprep; Nyegaard & Co., Oslo) gradients. After three washings in HBSS, the PBM or spleen cells were suspended in RPMI 1640 medium (Gibco) containing 15 percent fetal calf serum (Gibco). The adherent cells were removed by incubation for 1 hour at 37°C in petri dishes (Falcon; 60 by 15 mm) or by passage through nylon wool columns. Sheep red blood cells (SRBC) (Flow Laboratories) were washed three times in 0.1*M* phosphate-buffered saline (*p*H 7.2), and treated with neuraminidase (Behring Diagnostics) for 30 minutes. After treatment, the SRBC were suspended in HBSS containing 20 percent fetal calf serum preabsorbed with SRBC at a cell concentration of 1 percent (by volume). Equal volumes of lymphocyte suspension (5×10^6 cells per milliliter) and SRBC suspension were mixed and incubated for 5 minutes at 37°C, then centrifuged

for 5 minutes at 200*g* and incubated at 4°C for 2 to 18 hours. After incubation, the cells were gently resuspended and layered over Ficoll-Hypaque gradients. The SRBC rosette-forming cells (T cells) were found in the bottoms of the tubes after centrifugation. After lysis of the SRBC with tris-buffered ammonium chloride solution, the T cells were washed three times in HBSS.
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Cytoplasmic Reversion of *cms-S* in Maize: Association with a Transpositional Event

Abstract. *Spontaneous reversion to fertility in S male-sterile cytoplasm of maize is correlated with the disappearance of the mitochondrial plasmid-like DNA's, S-1 and S-2, and changes in the mitochondrial chromosomal DNA. Hybridization data indicate that one of the plasmid-like DNA's, S-2, is prominently involved in the mitochondrial DNA rearrangements.*

The S cytoplasm (*cms-S*) is one of several types of cytoplasmic male sterility in maize (*1*). *Cms-S* can be distinguished from other types on the bases of (i) certain nuclear genes called "restorers of fertility" (Rf) that suppress the expression of male sterility (*1*, *2*), (ii) electron microscopy and restriction endonuclease fragment analysis of mitochondrial DNA's (mtDNA's) (*3*, *4*), and (iii) the nature of mitochondrial protein products (*5*). In addition, two plasmid-like mtDNA's (S-1 and S-2) are uniquely associated with *cms-S* (*6*).

Certain *cms-S* strains undergo spontaneous reversion to male fertility (*2*, *7*). Genetic analysis of these revertants indicates that the underlying change can occur in either the cytoplasm or the nucleus. In this report we describe an analysis of the mtDNA's of seven cytoplasmically reverted strains. The results indicate that mitochondrial genes are involved in the cytoplasmic inheritance of male fertility. They also suggest that the unique plasmid-like DNA's identified in mitochondria of *cms-S* strains (*6*) are the physical manifestation of the fertility episome postulated earlier on the basis of genetic reversion analysis (*2*, *8*).

Concomitant with the reversion of the S type of male sterility to the fertile condition is the disappearance of the free plasmid-like DNA's, S-1 and S-2, and the appearance of new mtDNA restriction fragment patterns in the revertants. Significantly, we have demonstrated sequence homology between the plasmid-like DNA S-2, and some of the new restriction fragments. In other instances, S-2 homologies have been identified where new fragments were not visualized. It is clear that rearrangements have occurred in the mitochondrial chromosomal DNA and that sequences homologous to the S-2 DNA are prominently involved. It is tempting to speculate that the insertion of the plasmid-like sequences into the mitochondrial chromosome (or chromosomes) is associated with the reversion to male fertility.

Seven male-fertile revertant stocks were selected for study in which it was established by prior genetic analysis that the mutational event took place at the cytoplasmic level (*9*). The control strains, designated *cms-Vg* (M825/0h07), were derived from male-sterile siblings of revertants in families in which the male-sterile revertants were first identified. The standard *cms-S* control strain carried the nuclear background of inbred line B37.

Mitochondrial DNA's (*10*) from the standard *cms-S* source, a control *cms-Vg* (M825/0h07) strain, and two revertant strains, 285 and 369, were fractionated by agarose gel electrophoresis (Fig. 1). All cytoplasmic types exhibit an uppermost wide band containing the high-molecular-weight chromosomal mtDNA and a fast-migrating, low-molecular-weight (1.2×10^6) circular DNA (*6*). Two additional bands, designated S-1 and S-2, are present in mtDNA from the standard *cms-S* strain (Fig. 1A) and from

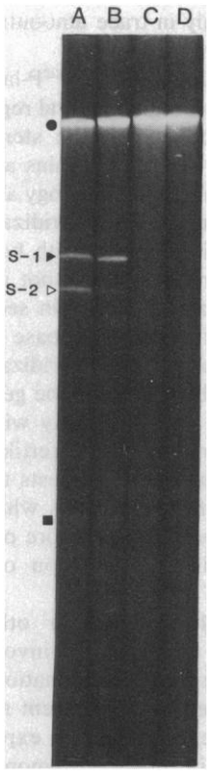

Fig. 1. Gel electrophoresis of mtDNA preparations from (A) *cms-S*, (B) *cms-Vg*, (C) revertant 285, and (D) revertant 369. Symbols: ●, high-molecular-weight chromosomal DNA; S-1 ▶, S-1 DNA; S-2 ▷, S-2 DNA; and ■, small-molecular-weight circular DNA.

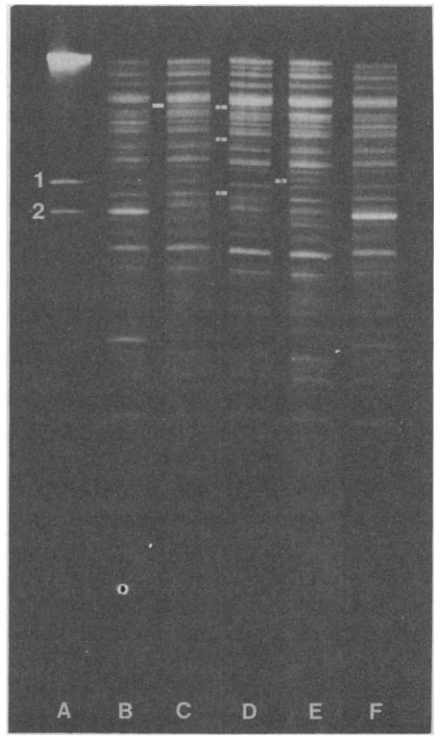

Fig. 2. Gel electrophoresis of *Xho*I digests of mtDNA from (B) *cms-S*, (C) revertant 251, (D) revertant 733, (E) revertant 369, and (F) *cms-Vg*; (A) is undigested mtDNA from *cms-S*, 1 is S-1 DNA, and 2 is S-2 DNA. Dashes indicate position of new bands in revertant strains which were absent in *cms-S* and *cms-Vg*.

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