otherwise normal, recipient mice. Strain (C3H/He × C57BL/Ha)F_1 male mice (12 weeks old) were exposed to 900 r of x-radiation and infused intravenously with 1.9 to 3.8 × 10^6 bone marrow cells taken from (i) (C3H × B10)F_1 mice of genotypes +/+, *Sl*/+, *Sl*^{d}/+, or *Sl*/*Sl*^{d} or (ii) (B10 × WB/Re)F_1 mice of genotypes *W*/+ or *W*^{v}/+. While progenitor cells of both *W*/+ and *W*^{v}/+ mice function deficiently (*4*), only *W*^{v}/+ mice are refractory to SFFV (*2*). Four weeks after transplantation, the marrow grafts of donor mice bearing one or more *Sl* mutant alleles did not confer refractoriness to SFFV to recipients (Table 2, *A*). However, there is some indication that *W*^{v}/+ marrow grafts rendered recipient mice more refractory to SFFV than did their *W*/+ counterparts (Table 2, *B*), although the difference is not significant ($P = .06$). These data suggest that progenitor cells of *Sl* mice are intrinsically susceptible to SFFV, while the opposite is probably true for progenitor cells of *W*^{v}/+ (and, presumably, *W*^{v}/*W*) mice.

If *Sl* anemic mice are refractory to SFFV because of an abnormal hemopoietic "environment" (*7*), then susceptibility to SFFV should *not* be transferable to such mice by normal (+/+) marrow cells. Preliminary experiments revealed that irradiated *Sl*/+ (unlike *Sl*/*Sl*^{d}) mice accepted marrow grafts like +/+ mice. Therefore, (C3H × B10)F_1 mice of genotypes +/+, *Sl*/+, and *Sl*^{d}/+ (8 weeks old) were exposed to 650 r of x-radiation and infused with 29.7 × 10^6 normal (+/+) (C3H/He × C57BL/Ha)F_1 marrow cells. Four weeks after transplantation, mice of *Sl*/+ and *Sl*^{d}/+ genotypes remained refractory to SFFV (Table 2, *C*). This result is in keeping with the abnormal environment hypothesis.

Since *Sl*/+, *Sl*^{d}/+, and *W*^{v}/+ mice are slightly anemic (*3*) and *Sl*^{T}/+ mice are normocytic, it is conceivable that anemia alone could have caused refractoriness to SFFV. Normal (C3H × C57BL/Ha)F_1 male mice (8 weeks old) were bled from their tail veins each day for 21 days (about 50 μl per day). Susceptibility to SFFV (BSB line) was determined after the last bleeding. Hematocrit values of the mice that were bled were 37 to 40 percent (normal is 42 to 48 percent). The spleens of these mice contained 0, 0, 1, 1, 1, 2, 3, 9, 9 (mean 2.9) foci, and the spleens of their nonanemic age controls contained 0, 0, 1, 2, 2, 3, 5, 5, 5, 6 (mean 2.9) foci. Thus, blood-loss anemia did not render mice refractory to SFFV.

Table 2. Susceptibility of mice to SFFV-BSB 4 weeks after x-irradiation and infusion of bone marrow cells. SFFV-BSB is the line of SFFV adapted to C57BL/Ha mice; all recipients received 12 focus-forming units as titrated in C57BL/Ha mice. Within group A, the mean counts from mutant donors were not significantly different from wild-type donors. In group B, the *P* value was .06; and in group C, the mean counts of mutant recipients were significantly lower ($P < .001$) than wild-type recipients. Results are means ± standard error; number of mice is given in parentheses.

Donor marrow		Recipient	
Genotype	No. cells infused (× 10^6)	Genotype	No. of foci per spleen
A. (C3H × B10)F$_1$ marrow cells injected into (C3H × C57BL)F$_1$ recipients			
+/+	1.98	+/+	4.7 ± 2.0(10)
Sl/*Sl*^{a}	1.96	+/+	6.1 ± 1.0(13)
+/+	3.21	+/+	6.3 ± 2.0(8)
Sl/+	3.78	+/+	3.1 ± 0.6(8)
Sl^a/+	3.38	+/+	2.8 ± 1.0(5)
B. (B10 × WB)F$_1$ marrow cells injected into (C3H × C57BL)F$_1$ recipients			
W/+	3.00	+/+	6.6 ± 3.4(7)
W^v/+	1.86	+/+	0.2 ± 0.1(12)
C. (C3H × C57BL)F$_1$ marrow cells injected into (C3H × B10)F$_1$ recipients			
+/+	29.7	+/+	6.7 ± 0.3(3)
+/+	29.7	*Sl*^{a}/+	0.0 ± 0.0(3)
+/+	29.7	*Sl*/+	0.3 ± 0.3(3)

In summary, the *Sl* locus affects the environment of SFFV target cells, so that they are either not available for infection or not able to form spleen foci once infected. Susceptibility to SFFV appears to be a sensitive indicator of hemopoietic function by progenitor cells. It is of interest to recognize that steps in normal differentiation and in neoplastic transformation can be influenced by the same genetic loci, by the nature of the hemopoietic environment, and by the nature of progenitor cells themselves.

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Reticuloendothelial Blockade: Effect of Puromycin on Opsonin-Dependent Recovery

Abstract. *Reticuloendothelial blockade induced by the administration of a gelatinized "reticuloendothelial test lipid emulsion" is due to a loss of opsonic activity in the plasma. Recovery from blockade, which is associated with restoration of plasma opsonins, was inhibited by the administration of puromycin. The effect of puromycin appears to be mediated by inhibition of opsonin formation rather than a puromycin-induced macrophage defect in phagocytosis.*

Particle-induced blockade of the reticuloendothelial system (RES) has been the subject of considerable investigation. Recent evidence has suggested that reticuloendothelial blockade is a manifestation of a loss of plasma opsonic activity, that is, loss of factors which promote phagocytosis (*1*) and not, as others have suggested (*2*), a direct saturation of the reticuloendothelial cells. If the blockade is a depletion of opsonic protein, then replen-

Table 1. Effect of puromycin on blockade recovery. Animals injected with blockading dose of "RE test lipid emulsion" alone, or with "RE test lipid emulsion" plus 15 mg of puromycin, or with 15 mg of puromycin. Half-time of clearance was determined 30 minutes after injection of the blockading dose for controls, and 180 minutes after the blockading dose for the others. Results are expressed as the mean ± S.E.M.

No. in group	Time after blockade induction (min)	Puromycin	$t/2$ (min)
	No blockade		
5			13.02 ± 1.21
4		+	18.38 ± 2.96*
	Blockade		
7	30		36.43 ± 3.71*
9	180		16.14 ± 1.59
6	180	+	31.07 ± 1.42*

* Significant ($P < .01$) when compared to controls which received no injections.

ishment of this protein should be associated with blockade recovery.

Fred and Shore (*3*) reported that puromycin had no effect upon the rate of intravascular clearance of a blockading dose of colloidal carbon. They suggested that synthesis of opsonic protein was not the rate-limiting factor in blockade, and proposed the mechanism of saturation of reticuloendothelial cell receptor sites.

In an attempt to ascertain whether recovery from the blockade is dependent on synthesis of opsonic protein or on a restoration of cellular receptor sites, we studied the influence of puromycin on the rate of recovery from the blockade and in the restoration of serum opsonic activity.

Initial experiments were made to assess the rate of recovery from reticuloendothelial blockade. Male Sprague-Dawley rats (250 to 300 g) were used. Blockade was established by the intravenous injection of gelatinized "RE test lipid emulsion" (*4*) at a dose of 50 mg per 100 grams of body weight. This dosage of lipid emulsion induces blockade of the RES (*1*). Intravascular clearance rates were determined with gelatinized ^{131}I-triolein labeled "RE test lipid emulsion" as described (*5*).

The half-time of clearance in normal untreated rats was approximately 12 minutes (Fig. 1). That the half-time for clearance of the test emulsion had increased nearly threefold 30 and 60 minutes after the blockading dose of lipid emulsion demonstrated induction of the so-called "blockade state." Recovery from blockade in the group of rats was evident first at 120 minutes and was nearly complete at 180 minutes.

In the evaluation of the influence of puromycin on recovery from RE blockade, animals were injected with a blockading dose of unlabeled lipid emulsion, as described, and were then immediately given intraperitoneal injections of 15 mg of puromycin in 2 ml of phosphate buffer (0.04*M*, *p*H 7.4). Control groups were given either lipid emulsion, puromycin, or phosphate buffer.

Rats given the blockading dose of lipid emulsion had a half-time clearance of 36 minutes when assessed 30 minutes after blockade (Table 1). By 180 minutes after blockade, the half-time values were again nearly normal in the control group. Puromycin effectively retarded recovery from blockade in that the mean half-time of clearance at 180 minutes was nearly identical to that of the group 30 minutes after blockade and increased twofold over the group not treated with puromycin. The reticuloendothelial depression observed in the puromycin-treated group was not due to the effects of puromycin, per se, since puromycin administration had only a slight depressant effect upon lipid emulsion clearance.

The demonstration that puromycin could effectively retard recovery from blockade suggested that protein synthesis was involved, since the dosage

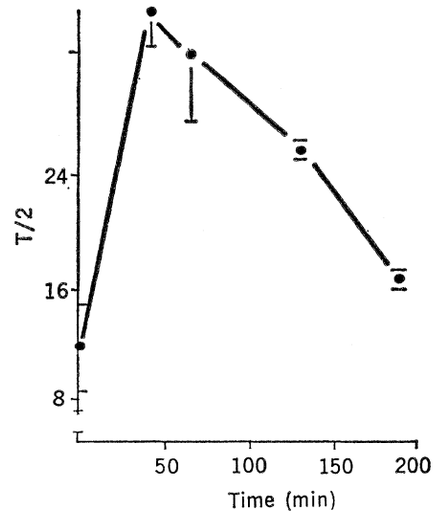

Fig. 1. Blockade recovery as a function of time. Intravascular clearance rates ($t/2$) determined at various times after injection of a blockading dose of "RE test lipid emulsion." Results are expressed as the mean ± S.E.M. of eight experiments.

of puromycin used effectively blocks protein synthesis in vivo (*3*). The question still remained whether synthesis of opsonic protein or of the cellular receptor site was involved in recovery from blockade. The latter possibility appeared unlikely, as no evidence had been reported which would suggest that the macrophage receptor site was a protein; indeed available evidence suggests a lipid moiety involvement (*6*). Nonetheless, these two possibilities were investigated with the use of the rat-liver-slice technique for the evaluation of macrophage and opsonic activity (*4*).

Liver slices were obtained from normal animals and from animals that received a blockading dose of "RE test lipid emulsion" and puromycin 180 minutes before the liver was extirpated. Serum was collected from similar groups of animals and from a group of rats that received puromycin alone.

The phagocytic activity of normal liver slices incubated with Krebs-Ringer phosphate buffer was negligible as indicated by the uptake of the emulsion, approximately 0.60 percent (Table 2). In the presence of normal serum, which contains opsonic activity, the uptake of the lipid emulsion by normal liver slices was increased 27-fold. In contrast, the incubation of normal liver slices in the presence of serum obtained from blockaded rats resulted in a 95 percent impairment in opsonic activity.

When liver slices derived from blockaded rats were incubated in the presence

Table 2. Effect of puromycin on phagocytosis-promoting activity of serum and on phagocytic activity of liver slices. Serum and slices were taken from untreated animals (normal) or from animals that had received a blockading dose of "RE test lipid emulsion" plus 15 mg of puromycin 180 minutes before removal. Results expressed as mean ± S.E.M.

Slice	Serum	Livers (No.)	Slices (No.)	Uptake (%/100 mg)
Normal	None	4	6	0.58 ± 0.05*
Normal	Normal	4	6	15.60 ± 2.40
Normal	Blockaded	6	13	1.08 ± 0.11*
Normal	Blockaded + puromycin	3	6	2.86 ± 0.73*
Blockaded	Normal	3	5	28.82 ± 3.02*
Blockaded + puromycin	Normal + puromycin	6	6	20.68 ± 2.78

* Significant ($P < .01$) when compared to normal liver slice plus normal serum.

of normal serum, there was no impairment in phagocytic activity, but, rather, a hyperphagocytic state was manifested. When liver slices derived from normal animals were incubated in the presence of serum obtained from puromycin-treated blockaded rats, there was an 82 percent reduction in the uptake of the emulsion. In contrast, liver slices from the blockaded puromycin-treated group, when incubated in normal serum, demonstrated no inhibition of particle uptake when compared to normal slices, evidence of intact hepatic macrophage activity.

These experiments suggest that the retardation of RES recovery from blockade by puromycin is attributable to an effect of puromycin upon synthesis of opsonic protein in the serum and not upon alterations of the phagocytic cell. Our data, in conjunction with that of Saba and Di Luzio (*1*), demonstrate that reticuloendothelial blockade is due to a depletion of opsonic activity in the serum. Furthermore, blockade recovery is attributable to a formation of opsonic protein, the synthesis of which can be readily impaired by puromycin.

The inability of Fred and Shore (*3*) to demonstrate an effect of puromycin upon clearance rates was probably due to the temporal relations between blockade recovery and opsonic restoration. The ability of liver slices from blockaded and from blockaded puromycin-treated animals to perform in the same manner as liver slices from normal animals demonstrates that blockade of the RES is not due to a cellular saturation (*2*) or to a receptor-site deficiency (*3*), but to a loss of opsonins which act as recognition factors (*4*) in the phagocytic event.

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Estrogen-Dependent Increase in Transfer RNA during Differentiation of the Chick Oviduct

Abstract. *Estrogenic hormones induce morphologic and biochemical differentiation in the oviduct of the immature chick. Concomitant with the hormone-stimulated tissue growth, there was an increase in 4S RNA, as judged by polyacrylamide-gel electrophoresis, and a corresponding increase in cellular transfer RNA activity, as measured by the amino acid acceptor capacity. This system may be suitable for studying the relation of hormones to transfer RNA in a differentiating tissue.*

Certain transfer RNA (tRNA) molecules may be involved in the regulation of protein synthesis and cell differentiation at the level of translation of messenger RNA (mRNA) (*1*). Changes have been reported in the relative amount of specific tRNA's of bacterial cells during sporulation (*2*), in phage-infected bacteria (*3*), in virus-infected animal cells (*4*), in differentiating wheat seedlings (*5*), and in developing chick erythrocytes (*6*), but there has been no report of a system in which a specific chemical stimulus induces synthesis of functional tRNA out of proportion to all other types of stable cellular RNA.

We have described the effects of estrogens such as diethylstilbestrol on morphologic and functional differentiation in the immature chick oviduct. After estrogen stimulation, three distinct types of epithelial cells differentiate from the apparently homogeneous population of primitive cells of the mucosa; and two of these cell types synthesize the cell-specific proteins ovalbumin and avidin which can be used as markers for differentiation (*7*). For these reasons, we have studied RNA patterns during different stages of hormone-induced biochemical and cytological differentiation in higher animals.

Three-day-old female Rhode Island Red chicks were injected daily with 5 mg of diethylstilbestrol subcutaneously for various periods up to 20 days. The animals were killed, and nuclear and cytoplasmic RNA fractions were prepared from the immature and estrogen-stimulated (differentiated) chick oviducts. The patterns were obtained with a recently described, sensitive technique for polyacrylamide-gel electrophoresis (*8*). The gels were stained with methylene blue and scanned with a densitometer (Photovolt). There was a fivefold increase or more in 4*S* RNA in both the nuclear and cytoplasmic RNA preparations of diethylstilbestrol-stimulated oviducts (Fig. 1). There were no qualitative changes in ribosomal RNA (30*S* and 18*S*) or other low molecular weight RNA's, and there was no evidence of degradation. Subsequent administration of progesterone

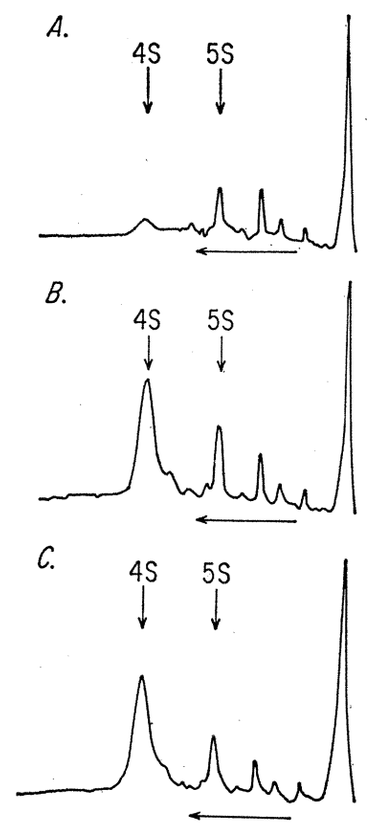

Fig. 1. Electrophoresis patterns of low molecular weight nuclear RNA extracted from oviducts of (A) immature chicks; (B) of chicks that received estrogen; and (C) of chicks that received estrogen and progesterone. The RNA samples were prepared as described (*8*); the direction of electrophoresis on 10 percent polyacrylamide gels (*8*) is designated by the arrow. The gels were stained with methylene blue (*8*) and scanned with a Photovolt densitometer.