Effect of Leucogenenol on Antibody Formation in Splenectomized Rats[1] (35622)

F. A. H. Rice and Richard Ciavarra[2]

Department of Chemistry, American University, Washington, D.C. 20016

Leucogenenol, a compound isolated by Rice (1) from the metabolic products of *Penicillium gilmanii* and by Rice and Shaikh (2) from bovine and human liver, stimulates the formation of stem cells (3) and increases the maturation rate of the myeloid series of blood cells (3, 4). Injection of leucogenenol hastens the regeneration of myeloid and lymphoid tissues damaged by X-radiation (5), and as a consequence sublethally X-irradiated mice that are injected with leucogenenol are capable of forming antibodies to sheep erythrocytes earlier than untreated mice (6).

Rabbits, rats, and mice (7, 8) are not capable of forming normal antibody titers to sheep erythrocytes for approximately a month after splenectomy. Taliaferro and Taliaferro (8) suggested that splenectomized animals cannot form normal antibody titers until other lymphoid tissues acquire the immunological function of the removed spleen.

It appeared, therefore, that the splenectomized animal could be used to determine whether leucogenenol affected normal as well as damaged lymphoid tissues. If leucogenenol affected normal as well as damaged lymphoid tissues, its injection should cause splenectomized animals to acquire the ability to produce antibodies to sheep erythrocytes earlier than usual.

We wish to report that 3 days after splenectomy rats that are injected with leucogenenol are capable of producing normal titers of hemolysin in response to the injection of sheep erythrocytes. In contrast, splenectomized rats not injected with leucogenenol do not show significant hemolysin titers after injection with sheep erythrocytes.

Materials and Methods. Twelve of 24 rats (Wistar, approximately 250 g in weight) were splenectomized and 3 days later each of the normal and splenectomized rats was injected intraperitoneally with 2×10^8 sheep erythrocytes suspended in 0.5 ml of pyrogen-free isotonic saline. At the time of injection with sheep erythrocytes and at 24-hr intervals for 6 days, each of six normal and six splenectomized rats were injected intravenously with 1 μg of the calcium salt of leucogenenol dissolved in 0.2 ml of pyrogen-free isotonic saline. Each of the remaining six normal and six splenectomized rats was injected with 0.2 ml of pyrogen-free isotonic saline at the time of injection with sheep erythrocytes and at 24-hr intervals for 6 days. On day 6 after injection with sheep erythrocytes each rat was exsanguinated by cardiac puncture under ether anesthesia and the blood so obtained (approximately 5 ml) allowed to clot at 5° overnight. The serum was then removed with a disposable Pasteur pipet, centrifuged at 8000 rpm for 10 min at 5°, and inactivated for 30 min at 56°. The hemolysin titer of the serum from each rat was determined by standard techniques (7, 9). Using the von Krogh equation, mean hemolysin $\log_{10}$ titers were calculated. Hemoglobin was determined on a Coleman-Hitachi spectrophotometer.

Results and Discussion. Table I shows the effect of leucogenenol on the mean hemolysin $\log_{10}$ titers of normal and splenectomized rats 6 days after they were injected with sheep erythrocytes. The mean titers of normal rats (-3.46 ± 0.92) are not affected by the daily injection of leucogeneol (-3.50 ± 0.92). However, rats that were injected 3 days after splenectomy with leucogenenol as

[1] Supported in part by Contract No. N00014-67-C-0275 with the Office of Naval Research Grant DA-ARO-49-092-65-686 from the Army Research Office, and a grant from The Lilly Research Laboratories, Indianapolis, Indiana.

[2] Graduate student, Department of Biology.

TABLE I. Effect of Leucogenenol on Hemolysin Titers of Normal and Splenectomized[a] Rats 6 Days after Injection with Sheep Erythrocytes.[b] Leucogenenol[c] was injected at the time of injection with sheep erythrocytes and at daily intervals thereafter for 6 days.

	Mean hemolysin log titers ± standard error[d] (log_{10})	
	Controls (not injected with leucogenenol)	Injected with leucogenenol
Normal	−3.46 ± 0.92	−3.50 ± 0.92
Splenectomized	−1.55 ± 0.92	−3.52 ± 0.30

[a] Rats were injected 3 days after splenectomy.

[b] Sheep erythrocytes (2×10^8) were suspended in 0.5 ml of pyrogen-free isotonic saline and injected intraperitoneally.

[c] Rats were injected intravenously with 1 μg of the calcium salt of leucogenenol dissolved in 0.2 ml of pyrogen-free isotonic saline. Control animals were injected intravenously with 0.2 ml of pyrogen-free isotonic saline.

[d] Standard errors were calculated from the results on six animals.

well as sheep erythrocytes and daily thereafter with leucogenenol show an increase in the mean hemolysin log_{10} titer from −1.55 ± 0.92 (not injected with leucogenenol) to normal titers of −3.52 ± 0.30 (injected with leucogenenol) 6 days after they were injected with sheep erythrocytes. Hemolysin titers of normal and splenectomized rats that were not injected with leucogenenol are in agreement with the titers obtained by Rowley (10).

It would appear that the injection of leucogenenol increases the rate at which cells that are potentially capable of antibody production but are not operative in normal animals assume the function of similar active cells that are removed at splenectomy.

The fact that injection of leucogenenol does not increase the hemolysin titers of normal rats could result from the operation of the feedback regulation of the immune response suggested by Schier and Schwartz (11) and Moller and Wigzell (12). It has also been reported that antibody synthesis is suppressed by the same antibody already present in the animal (13, 14). If hemolysin titers are determined by a feedback mechanism, then in the normal animal an increase in the number of cells capable of antibody production would not necessarily result in increased antibody titers. In the splenectomized animal leucogenenol, by stimulating the formation of cells capable of antibody synthesis, might be expected to decrease the time required for the animal to recover normal immunological competency. However, the resulting hemolysin titers would be normal if they were determined by a feedback mechanism. The early period at which splenectomized animals treated with leucogenenol recover immunological competency, together with the observation that leucogenenol increases the maturation rate of blood cells (3–5), suggest that it increases the rate of transformation of precursors into cells capable of antibody synthesis.

Summary. Three days after splenectomy rats unable to form normal hemolysin titers to sheep erythrocytes will produce normal titers when they are injected with leucogenenol as well as sheep erythrocytes. Normal rats do not respond with an increase in hemolysin titers when they are injected with leucogenenol as well as sheep erythrocytes. The injection of leucogenenol apparently stimulates cells that are potentially capable of antibody synthesis.

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Received Jan. 15, 1971. P.S.E.B.M., 1971, Vol. 137.