variances overall, the probability of an error in the rejection of the hypothesis when it should have been accepted would be questionable for the tests on the means in an analysis of variance; therefore the data for individuals were not pooled or averaged. The sums for all individuals per time period per day appear graphically in Fig. 2; sums of several individuals should more validly represent a possible rhythm in secretion for *L. longipes* than the value obtained from one individual because of the great individual variation, particularly during periods of peak activity.

The method used to measure 5-HT required the use of entire brains and guts for analysis, so a 24-hour rhythm of secretion for a single individual was unobtainable. Other investigators have found wide variation between individuals as to the time of occurrence of a specific secretion response. Beck *et al.* (*8*), in studying cornborers, found that the probability of an arbitrarily chosen individual showing a secretion response within a 2-hour interval was approximately 67 percent; this figure may be even lower in quantitative studies. Tissues in vitro secreted less total 5-HT than those in vivo. The mass of tissue after 80 hours in vitro was about 25 percent of its original volume.

A technique has been developed for maintaining functionally active tissues of *L. longipes* for long periods. These tissues showed rhythmic secretions. Comparison of the activities of tissues in vitro and in vivo showed similarity in secretion patterns regarding times of greatest activity. However, the in vitro secretion was bimodal; the in vivo unimodal. The cultured tissues exhibited a secondary peak of activity in secretion at a time when production was lowest in vivo. Such results suggest endogenous control of secretion of 5-HT, and a possible central mechanism of feedback control. Is the secretion of 5-HT controlled by photoperiod in this arachnid as it appears to be in vertebrates (*4*)? The phenomenon of cyclic secretion has been noted in the endocrine glands of vetebrates briefly held in vitro (*9*). Confirmation of cyclic activities in tissues long maintained in culture would interest investigators of the nature of the biological clock. Our technique would be useful for examining the question of exogenous versus endogenous control of cyclic phenomena.

Table 1. Sums of 5-HT from 36 brains and 36 guts after about 80 days in vitro. Every 4 hours during a 24-hour period six brains and six guts were removed from Falcon flasks; each sample is a pool of two brains and two guts. Lack of homogeneity of variance prevented statistical handling of the data.

Time (hours)	5-HT ($\mu\mu$mole)	
	In three samples	Total
1000	18.9, 18.9, 18.9	58.7
1400	12.1, 12.1, 12.1	36.3
1800	6.8, 4.6, 2.3	15.9
2200	9.4, 19.0, 28.1	56.5
0200	20.0, 23.6, 27.3	70.9
0600	0 0 0	

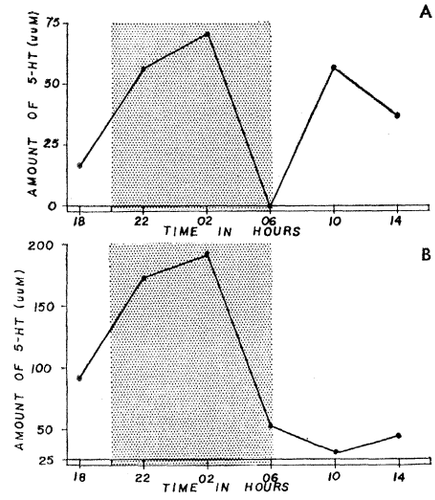

Fig. 2. Daily rhythmicity of the secretion of 5-HT in tissues in vitro. The pattern of secretion is rhythmic, peaking at 0200 hours as in vivo. However, the overall 24-hour pattern is bimodal, with a second peak at 1000 hours (midmorning)—a time when the pattern in vivo is at its lowest point. *A*, In vitro data; *B*, in vivo data for day 1; the curve is representative of all data obtained and is used here for comparison. The stippled areas indicate night during the 24-hour cycles.

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Heme Stimulation of Globin Synthesis in a Cell Free System

Abstract. *In a cell-free system of pigeon erythrocyte nuclei high concentrations of oxygen inhibit globin synthesis because of the inhibition of heme synthesis, which is required for globin synthesis. The effect of heme in overcoming the inhibition of globin synthesis by oxygen is manifested only during the first few minutes of incubation. This period represents the time during which the system is being warmed from 4° to 37°C. The effect of heme seems to be upon some initial assembly or structural process.*

The nuclei of avian erythrocytes carry out the synthesis of globin (*1*, *2*). In cell-free preparations of these nuclei the synthesis of globin is stimulated by heme and by hypoxia (*3*, *4*). This reversible inhibition of hemoglobin synthesis by oxygen is relieved by the addition of heme (*4*). Falk and Porra (*5*) have shown that oxygen limits heme synthesis by inhibiting the formation of uroporphyrinogen from porphobilinogen.

On the basis of these findings, a model was proposed for a direct biochemical regulation of hemoglobin synthesis in which hypoxia stimulates the synthesis of heme which, in turn, increases the rate of globin synthesis (*4*). Further studies on the kinetics of this regulatory process are in agreement with the proposed model and suggest a mechanism of action of heme on protein synthesis.

Nuclei were isolated from pigeon red cells hemolyzed with 0.5 percent saponin in a solution containing 0.25*M* sucrose, 0.003*M* $CaCl_2$, and $10^{-5}M$ ferric ammonium sulfate and centrifuged at 1000*g*. The washed nuclei were incubated in 5-ml serum vials in a medium containing 0.02*M* sodium phosphate buffer, 0.015*M* KCl, 0.03*M* glucose, and 9.6×10^{-4} mmole (0.25 μc) of L-leucine-C^{14} (uniformly labeled) per vial. The vials were closed with rubber-dam stoppers and metal caps and flushed with the various gas mixtures. All of the above procedures were performed while the vials were packed in shaved ice. Incubations were at 37°C in a Dubnoff metabolic shaker. The reactions were stopped by placing the vials in ice and injecting through the rubber dam 1 ml of 10 percent trichloroacetic acid (TCA). The TCA precipitates were purified

and plated, and the radioactivity was determined by counting (*3*).

Hemin (recrystallized, Nutritional Biochemicals Corporation) was dissolved in a tris-buffered solution and used in a concentration of 150 μg per milliliter of incubation mixture (*2, 4*).

Hyperoxia inhibits protein synthesis, but the rate of incorporation is returned to the level observed at 10 percent oxygen by the addition of heme (Fig. 1). During the first 20 minutes of incubation endogenous heme synthesis apparently is the only limiting factor for protein synthesis since added heme is no more effective in overcoming the inhibition by oxygen than is hypoxia alone. An oxygen concentration of from 5 to 15 percent is the optimum for heme and globin synthesis in this system (*4*). Therefore, when endogenous heme synthesis is maximum (at 10 percent oxygen), added heme has little or no effect on globin synthesis. When endogenous heme synthesis is limiting (at 100 percent oxygen) the addition of heme restores the optimum rate.

Table 1. Effect of delayed addition of heme on incorporation of C^{14}-leucine into protein. Nuclei were incubated in an atmosphere of air.

Time of addition (min)	Radioactivity at 30 min (count min^{-1} mg^{-1})
No addition	
0	161
Heme added	
0	262
1	257
2	230
3	204
5	185
15	160
Heme + C^{14}-leucine + other amino acids	
5	171
15	161

After 30 minutes of incubation the rates of incorporation in the presence of 10 percent oxygen alone and 100 percent oxygen alone, or with heme, gradually become identical, an indication that a factor other than hypoxia-induced heme synthesis has become the limiting factor for protein synthesis. Heme continues to exert a protective effect on protein synthesis over and above that of hypoxia alone.

Heme exerts its stimulatory effect by prolonging the initial linear rate of incorporation (Fig. 1). Calculations from several kinetic experiments show that the increase in protein synthesis with heme is proportional to the degree of prolongation of the initial rate of incorporation, an indication that heme acts upon some initial step in protein synthesis rather than upon rate of synthesis itself.

Further support for this notion comes from the fact that the capacity of heme to stimulate protein synthesis depends upon its being present during the early minutes of incubation at 37°C. In an experiment in which nuclei were incubated in air with C^{14}-leucine and heme was added at intervals during the first 15 minutes of incubation (Table 1), incorporation was greatest when heme was added at zero time, and the stimulatory effect on protein synthesis diminished progressively with the later additions of heme. Heme also failed to stimulate when added after 10 minutes in both 10- and 100-percent oxygen atmospheres. The effect of heme was the same whether or not labeled amino acid was added simultaneously with heme, and this indicates that it probably does not act by stimulating amino acid activation.

The loss of the capacity to respond to heme is temperature dependent.

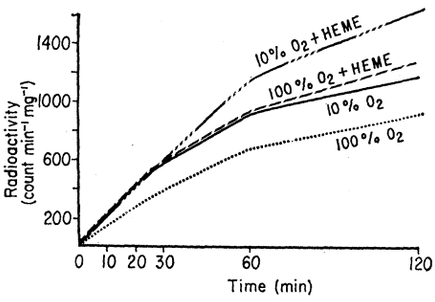

Fig. 1. Effect of heme on incorporation of C^{14}-leucine into protein during hypoxia and hyperoxia.

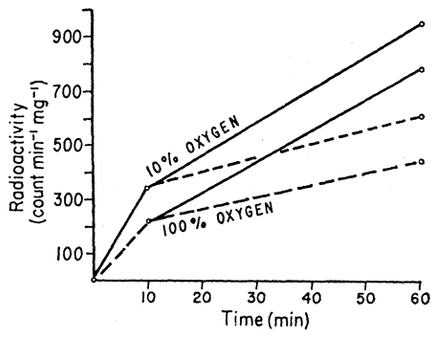

Fig. 2. Effect of changing the oxygen tension in the atmosphere on the incorporation of C^{14}-leucine into protein. At 10 minutes the atmosphere was changed in half of the vials. Solid lines represent 10 percent oxygen and dotted lines represent 100 percent oxygen.

Table 2. Effect of delayed addition of heme on incorporation of C^{14}-leucine into protein at different temperatures. Nuclei were first incubated at the temperatures indicated. The reactions were stopped by the addition of trichloroacetic acid at 60 minutes.

Time of addition (min)	Activity (count min^{-1} mg^{-1})							
	Expt. I		Expt. II		Expt. III		Expt. IV	
	10°C	37°C	12°C	37°C	15°C	37°C	20°C	37°C
				Control				
		395		253		997		137
				Heme added				
0		653		493		1520		180
3			351	300	1300	1115	174	
10	560	429			1170		144	147
15		440	354	215		940		149
20	485				1000			
30	509		320					
40	467							

Nuclei were incubated in an air atmosphere for varying lengths of time at different temperatures before the addition of heme, and afterward incubation was continued for 60 minutes at 37°C. At 10° to 12°C heme was effective for at least 30 to 40 minutes. Above this temperature the heme effect was lost after 10 minutes of the prior incubation and recooling to 0°C did not restore it. Therefore, the inactivation of some step in protein synthesis occurring in the initial minutes of incubation at 37°C may be delayed or prevented by cooling or by the addition of heme. In addition, since heme did not prevent the inhibition of protein synthesis by puromycin, cycloheximide, or actinomycin, it seems likely that it acts on the assembly of the ribosomal system, the action taking place as the incubation mixture is warmed to 37°C. These kinetic data are in agreement with the report by Waxman and Rabinovitz that heme regulates the rate of globin synthesis in rabbit reticulocytes by "stabilizing" synthetically active ribosomes (*6*).

In contrast to the heme effect, changing the oxygen tension after 10 minutes of incubation at 37°C changes the rate of protein synthesis (Fig. 2). This rate change occurs both in the presence and absence of heme, and it indicates that oxygen exerts some effect on the actual rate of protein synthesis independent of the action of heme. The fact that changes in oxygen tension affect the synthesis of globin after the first incubation, even when heme no longer is effective, indicates that the protective effect of heme in preventing peroxide accumulation cannot be exerted through its catalase activity. The fact that heme relieves completely the inhibition by oxygen during the early minutes of incubation, returning the rate of protein synthesis to that during hypoxia, which is maximal for heme synthesis, supports the regulatory mechanism proposed, namely that oxygen inhibits globin synthesis by inhibiting the formation of heme.

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Virus-induced Murine Leukemia: Its Inhibition and Suppression by Serum Containing Erythropoietin

Abstract. *Mice infected at 2 days of age with a virus that induces reticulum cell sarcoma and myeloerythroleukemia were treated with erythropoietin-containing serum obtained from rabbits that had been treated with acetylphenylhydrazine. A marked inhibition of tumor induction occurred, particularly in females, when treatment was begun early. Delayed treatment resulted in instances of regression of overt neoplasia.*

Certain experimental findings, of our own and others, suggested to us the advisability of investigating the effect of erythropoietin on a virus-induced leukemia which we described a few years ago (*1*). Foremost among these findings was that abnormal erythroid proliferation is a prominent feature of this leukemia; this finding suggested that the disease could be due, in part, to a disturbance in erythropoiesis. A second finding was that the disease is sex-oriented, males being at least twice as resistant as females. Moreover, castration diminished the natural resistance of males; in a study involving 95 male MABA mice, the incidence of disease in castrates was more than twice that in intact animals. Further, administration of testosterone altered the natural susceptibility of females; in a study involving 72 female MABA mice, the incidence of disease in untreated females was nearly twice that in treated ones. These findings concerned with our disease, together with two reports in the literature concerned with erythropoiesis, focused our attention on the possibility that the difference in susceptibility of the sexes to the disease could have resulted from a difference in their normal levels of erythropoietin. The experiments in the literature were those of Fried, De Gowin, and Gurney (*2*), who discussed the erythropoietic effect of testosterone in mice, and those of Mirand, Gordon, and Wenig (*3*), who attributed the effect of testosterone to stimulation of erythropoietin.

Erythropoietin-containing serum (ERS) was prepared as follows: New Zealand White rabbits, in groups of six, were inoculated subcutaneously with a 2½ percent solution of 1-acetyl-2-phenylhydrazine, 0.35 ml per pound, one dose daily for four consecutive days. On the 5th day surviving animals were exsanguinated. Serums from rabbits with hematocrits of 15 percent by volume, or less, were pooled and frozen at −20°C until use. Two such pools were assayed for erythropoietin by an established procedure (*4*). One pool, assayed after 6 months in storage, was estimated to contain 8 to 10 units per milliliter of serum; a more recent pool assayed 5.6 units per milliliter. When normal rabbit serum (NRS) was required, it was obtained from rabbits which had not, to our knowledge, been previously bled.

In our experiments inbred BALB/c mice maintained in our colony were randomly bred. First-generation (F_1) mice were inoculated both subcutaneously and intraperitoneally at 2 days of age with an extract of 10 percent neoplastic spleen containing virus; 0.05 ml was given at each site. The animals were weaned at 5 to 6 weeks of age, at which time weekly examinations were begun to determine the progress of their disease. The examination consisted primarily of palpation of the spleen and inguinal lymph nodes. Numbers from "0" to "3.5" were assigned to the mice, corresponding to the development of disease. Number "2" represented, in an adult mouse, a spleen of approximately 500 mg (normally about 125 mg) and enlarged lymph nodes. These were considered to be unequivocal signs of neoplastic disease. Number "3" represented an advanced case in which the spleen weighed 1000 mg or more. The disease in an animal was considered to have regressed if, after progressing to at least the stage "2", it regressed to "1" or less. In most instances, these limits were exceeded.

In the first experiment, treatment of mice (primarily BALB/c females) with ERS commenced at weaning. It consisted of the subcutaneous inoculation of 0.5 ml of serum per mouse weekly for 8 weeks. At first the results seemed disappointing, for we anticipated some effect of the treatment on the incidence of leukemia rather than on the disease after it had become overt. Indeed, the incidence (number neoplastic/number inoculated) was initially somewhat higher (22/25) and the latency somewhat reduced (107 days) in the treated mice compared with the controls (18/25, 126 days), but in the course of subsequent weekly examinations it became evident that palpable lesions in treated mice had regressed in ten instances. Five mice with regressed lesions were killed at 207 days. One had been a "3" at 120, had regressed to "1" at 183 days, and had become a "2" again; two had been "2.5" at 110 days, regressing to "0" at 161 and 187 days, respectively, where they remained until sacrifice;