Technical Papers

The Effect of B-Pyracin and the *Lactobacillus casei* Factor Upon Hemoglobin Regeneration Following Hemorrhage[1,2]

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Recently Scott, Norris, Heuser, and Bruce (*4*) reported that the macrocytic hypochromic anemia which develops in chicks fed a purified diet is prevented by administration of the crystalline *Lactobacillus casei* factor (LCF) of Hutchings, Stokstad, Bohonos, and Slobodkin (*2*) together with either the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine (α-pyracin lactone) or the isomeric 4-carboxy lactone (β-pyracin lactone).[3] Kornberg, Tabor, and Sebrell (*3*) have shown that the crystalline LCF of Hutchings and associates (*2*) has a preventive and corrective action on hemorrhagic anemia in rats fed a purified diet containing succinyl sulfathiazole.

Since, in the chick, LCF and pyracin appear to be concerned in hematopoiesis under conditions of malnutrition, the question arose as to whether or not injections of these factors might speed up hematopoiesis in times of great need, as for instance, after severe hemorrhage, even when the subjects are receiving a good diet. Experiments were conducted, therefore, to determine if it is possible to hasten hemoglobin regeneration, following severe hemorrhage, by intramuscular injections of LCF and β-pyracin. The results of these experiments are presented in this report.

Three different experiments were conducted using a total of 34 White Leghorn hens in active egg production. Each hen was kept in an individual wire cage in a heated room and fed a commercial diet throughout the experiment. The hens were rendered anemic by withdrawal of blood from the heart with a Luer's syringe. The amount withdrawn was calculated to be approximately one-third of the hen's total blood supply. The blood withdrawal had no effect upon the rate of egg production.

After bleeding, the hens in each experiment were separated into four groups. Group 1 acted as the control and received no therapeutic treatment. Group 2 received 50 γ of β-pyracin[4] per hen, by intramuscular injection, 24 hours after bleeding and subsequently every 24 hours until termination of Experiments 1 and 2, and until the fourteenth day of Experiment 3. Similarly, Group 3 received 50 γ of LCF,[5] and Group 4 received 50 γ each of β-pyracin and LCF per hen per day.

Hemoglobin values were determined for each hen before the experiments were started. Hemoglobin determinations were also made just prior to injection, 24 hours after bleeding, and, in like manner, daily thereafter until the injections were discontinued.

TABLE 1

HEMOGLOBIN VALUES OBTAINED IN HENS ADMINISTERED B-PYRACIN AND *Lactobacillus casei* FACTOR (LCF) AFTER HEMORRHAGE

Treatment	No. hens per lot	Hemoglobin before bleeding grams/100 cc.	Hemoglobin after bleeding by 24-hour intervals grams/100 cc.								
			1	2	3	4	5	6	7	8	9
Experiments 1 & 2 (Preliminary)											
Control ...	6	8.6	5.7	6.4	5.6	6.2	6.4	6.7	6.8	7.3	..
β-pyracin .	5	8.2	5.7	5.6	6.2	6.8	7.0	8.0	7.7	8.2	..
LCF	5	8.1	5.9	6.2	6.6	6.5	7.1	7.6	7.9	8.5	..
β-pyracin + LCF ...	6	8.6	5.7	6.1	6.6	7.4	7.8	9.0	9.4	9.7	..
Experiment 3											
Control ...	3	7.5	4.5	4.4	4.8	4.2	5.4	5.6	5.3	5.8	6.0
β-pyracin .	3	7.5	4.6	5.6	6.5	7.1	6.8	6.7	6.5	8.1	8.0
LCF	3	7.7	4.9	5.6	5.9	6.4	6.4	7.5	7.2	7.9	7.5
β-pyracin + LCF ...	3	7.9	4.9	5.6	6.1	7.1	7.3	8.1	8.3	8.9	9.7

The "pre-bleeding" hemoglobin values obtained on these experiments were lower than the value of 9.8 grams per 100 cc. of blood reported to be normal for the hen by Dukes and Schwarte (*1*). We have obtained values on hens receiving the same diet, but not in active egg production, which are in agreement with the value reported by these workers.

[1] This work was aided by grants to Cornell University by the Western Condensing Company, San Francisco, California, and the Wyeth Institute of Applied Biochemistry, Philadelphia, Pennsylvania, and was conducted in the nutrition laboratories of the Department of Poultry Husbandry. The paper was submitted in July 1945.

[2] The technical assistance of R. F. Ball is gratefully acknowledged.

[3] Unpublished studies conducted in this laboratory, together with the data presented here in Experiment 3, show that both the acid and the lactone forms of these compounds are active, nutritionally. Therefore, due to its connotation, the name pyracin will henceforth be applied to the acid forms, while the lactone forms will be referred to as pyracin lactones.

[4] We are indebted by Merck and Company, Rahway, New Jersey, for the crystalline β-pyracin lactone used in Experiments 1 and 2. The lactone was converted to β-pyracin for use in Experiment 3 by refluxing 5 mg. in 20 cc. of 0.05N NaOH for 15 minutes.

[5] We are indebted to Dr. E. L. R. Stokstad and Dr. B. L. Hutchings, of Lederle Laboratories, Pearl River, New York, for the crystalline *Lactobacillus casei* factor used in these studies.

The results of the hemoglobin determinations which are presented in Table 1 show that the administration of either β-pyracin or LCF alone is of some value in hastening the regeneration of hemoglobin after severe hemorrhage, but that the administration of both these factors together is markedly more effective, causing a return of the hemoglobin level to the "pre-bleeding" value in five to six days, whereas in the hens receiving no treatment only a slight increase in hemoglobin level was observed in that length of time. Furthermore, the hemoglobin level in the hens receiving both β-pyracin and LCF continued to rise in all experiments and reached the normal level in eight to nine days, while the controls showed an increase in hemoglobin of approximately 1.5 grams per 100 cc. of blood in the same period of time.

In Experiment 3, the hens were held after the vitamin injections were discontinued and periodic hemoglobin determinations were made in order to ascertain the ultimate effect of the removal of approximately one-third of the blood supply. The results are presented in Fig. 1. The results show that in all cases

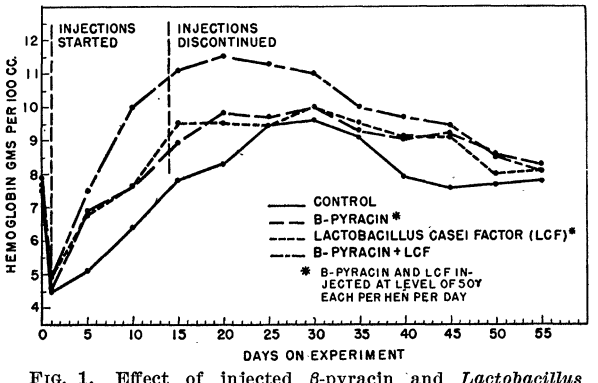

FIG. 1. Effect of injected β-pyracin and *Lactobacillus casei* factor upon the rate of recovery from hemorrhagic anemia.

the hemoglobin levels rose above the "pre-bleeding" values and then declined to approximately the original level. The rate of increase was much more rapid and the level reached was higher in the lot receiving both β-pyracin and LCF than in the other lots. In this lot and the lots receiving β-pyracin and LCF singly, the higher hemoglobin levels were maintained for some time before declining. The rise in hemoglobin levels above the "pre-bleeding" values, which was observed in all lots, may possibly be explained by an overcompensatory activity of the hematopoietic organs under conditions of extreme demand.

SUMMARY

In severe experimental hemorrhagic anemia in hens, the injection of β-pyracin and *Lactobacillus casei* factor, alone and in combination, was found to hasten the regeneration of hemoglobin. When these factors were administered together, however, the rise in hemoglobin was much more rapid and the level reached was higher than in the lots administered the factors alone.

The hemoglobin levels in the control lots rose above the "pre-bleeding" values and then began an almost immediate decline to approximately the original level. In the lots receiving β-pyracin and *Lactobacillus casei* factor, alone or in combination, the hemoglobin levels also rose above the "pre-bleeding" values but were maintained for some time after injections were discontinued before declining to the original values.

References

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The Site of Action of DDT in the Cockroach[1]

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Yeager and Munson (*4*) have obtained indirect evidence that DDT (*1*-trichloro-2,2-bis(*p*-chlorophenyl)-ethane) may act on motor nerve fibers of the cockroach, raising their irritability until spontaneous discharges result in muscle twitches. Others have confirmed this, observing the twitching which occurs in amputated DDT-treated legs.

If an emulsion[2] containing .5 μg. DDT is injected into the body cavity of an adult cockroach (*Periplaneta americana*) weighing 1.0 to 1.5 grams, the insect shows typical symptoms of poisoning within 5 or 10 minutes. Briefly, the symptoms consist of coarse clonic spasms of the trunk and appendicular muscles and increased but initially well-coordinated activity. Within a few hours the spasms become continuous and the insect is unable to stand, although the legs may continue to twitch feebly for 48 hours.

The dose producing tremors within 5 to 10 minutes is in the neighborhood of 5 to 10 mg./kg. If it is assumed that DDT is distributed evenly among the body tissues, the toxic concentration at the site of action must be approximately 5 parts per million. Even if DDT is selectively accumulated in certain

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Tufts College.

[2] Twenty ml. of an oil solution (petrolatum mineral oil, 90.5 per cent; oleic acid, 8.5 per cent; and DDT, 1.0 per cent) is passed through a hand emulsifier with 80 ml. of an aqueous solution (insect saline, 99.0 per cent; triethanolamine, 1.0 per cent). The emulsion minus DDT had no effects on cockroaches.