Biol. & Med., 1961, v107, 887.

5. Jacobson, L. O., Marks, E. K., Gaston, E. O., Simmons, E. L., Block, M. H., Science, 1948, v107, 248.

6. Stohlman, F., Jr., Cronkite, T. P., Brecher, G., Proc. Soc. Exp. Biol. & Med., 1955, v88, 402.

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Erythropoietin-Like Effect of a Polycythemic Virus.* (32146)

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In a hypertransfused-polycythemic (HP) state erythropoietin production and morphological evidences of erythropoiesis disappear(1,2). The only substance reported to date that can initiate erythropoiesis in a HP state is erythropoietin, whether administered exogenously or produced through the effects of other substances(1-4). Consequently, the HP state has been used as a reliable assay system for detection of erythropoietin activity in plasma, urine, and other body fluids. In the course of working with a murine virus causing polycythemia(5), we noted the ability of this virus to initiate erythropoiesis in HP mice. The purpose of this communication is to report our findings on the ability of this erythropoietic virus to initiate erythropoiesis in a HP state.

Material and methods. Animals. Ha/ICR Swiss mice, 5-6 weeks old, were used throughout the investigation. They were fed pellets of Derwood-Morris Diet (TEKLAD) and water was available to them at all times.

Hypertransfused-polycythemic (HP) state. The HP state was induced in the mice by giving intraperitoneal injections of 0.5 ml of washed, packed, homologous red blood cells on 3 consecutive days and repeated on day 5(1). This resulted in hematocrits in the vicinity of 70-75%. The virus was given to each group when the HP state was achieved. The HP state in infected mice was prolonged by giving red cell transfusions twice a week to maintain the hematocrit above 70%.

Virus. The polycythemic virus, which could be a variant of Friend virus(6) or a passenger virus(5), present in spleen filtrate prepared originally from Friend virus infected Taconic Swiss, has been passed in our laboratory since 1961 and it is in its 96th passage generation. The erythropoietic pattern induced by Friend virus as originally reported by Friend(6) and that induced by the polycythemic virus is considerably different. Friend virus does not produce polycythemia, increase in granulocytes or an increase in platelets.

The mice in the experiments described herein were inoculated intraperitoneally with 0.2 ml of filtrate made from spleens of animals infected with the polycythemic virus. The titer of the 10% splenic filtrate was $10^{4.5}$ ID_{50}/ml.

Erythropoietic parameters. Fe_{59} uptakes(7) and reticulocyte counts were used primarily to determine erythropoiesis. All mice were injected intravenously with 0.5 μc of Fe_{59} at various intervals following virus infection and Fe_{59} uptake in the femur, blood, and spleen was determined 24 hours later.

Erythropoietin assay. HP mice were used for assaying the presence of erythropoietin in plasma and urine of mice infected with virus(8). Plasma and urine were inactivated for virus activity by subjecting the plasma and urine to 56°C for 30 minutes.

Results. Characteristics of hypertransfused-polycythemic (HP) state (Table I): Attention should be directed to the characteristics of erythropoiesis one encounters in the HP mouse in order to understand the effect the polycythemic virus has in this state.

In the HP mouse all evidence of active erythropoiesis in the bone marrow, blood,

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and spleen ceases, as substantiated by the low Fe_{59} uptakes shown in Table I (compare Groups a and b). Also, the peripheral reticulocyte counts are 0.00%. Moreover, pro-erythroblasts and erythroblasts in the bone marrow and spleen are markedly depressed. From these data one can see how effectively hypertransfusion with washed red blood cells can inhibit erythropoiesis. Administration of exogenous erythropoietin can initiate erythropoiesis in this state (compare Groups b and c).

Response of normal mice and HP mice to polycythemic virus (Table II). *Normal mice injected with polycythemic virus.* Splenomegaly resulted as the disease progressed in time in normal mice given the polycythemic virus. The spleen increased in weight: 0.89 g at 7 days, 1.24 g at 14 days, 2.98 g at 21 days, and 3.42 g at 28 days. Histological examinations of the spleens throughout the investigation revealed a progressive reticulum

TABLE I. Some Characteristics of a Hypertransfused-Polycythemic (HP) State in Ha/ICR Swiss Mice.

Group	Avg 24 hr Fe_{59} uptake*			Avg ht	% Reticulocytes
	Femur	Blood	Spleen		
(a) Normal mice	.49 ± .05	24.8 ± 2.8	1.2 ± .08	42 ± 1.2	0.5
(b) HP mice + saline	.09 ± .02	.41 ± .09	.07 ± .03	75 ± 3.4	0.00
(c) HP mice + erythropoietin†	.54 ± .09	19.8 ± 3.4	5.8 ± 1.2	73 ± 2.8	1.8

* Average of 5 animals.
† One unit of erythropoietin administered s.c. on 3 successive days and, on the 4th day, 0.5 μc Fe_{59} injected i.v. Fe_{59} uptakes determined on the 5th day.
± = Standard deviation.

TABLE II. Response of Normal (N) and Hypertransfused-Polycythemic (HP) Ha/ICR Swiss Mice to Polycythemic Virus (PV).

Group	Days post-infection			
	7	14	21	28
Non-infected normal (N)				
Avg 24 hr Fe_{59} uptake*—Femur	.49 ± .08	.47 ± .05	.44 ± .08	.50 ± .08
Blood	25.6 ± 2.4	24.8 ± 2.2	23.8 ± 3.2	25.2 ± 2.4
Spleen	1.2 ± .42	1.1 ± .09	1.1 ± .09	1.9 ± .04
Avg spleen wt (g)	.10 ± .06	.12 ± .03	.12 ± .04	.17 ± .05
Avg ht	41 ± 2.1	40 ± 1.2	42 ± 1.2	41 ± 1.1
N + PV				
Avg 24 hr Fe_{59} uptake*—Femur	.42 ± .07	.10 ± .03	.12 ± .03	.08 ± .01
Blood	27.4 ± 2.2	29.0 ± 3.1	30.4 ± 4.2	31.2 ± 1.4
Spleen	6.9 ± 1.2	18.6 ± 1.2	21.2 ± 3.4	18.9 ± 2.9
Avg spleen wt (g)	.89 ± .12	1.24 ± .07	2.98 ± .59	3.42 ± 1.01
Avg ht	49 ± 3.1	57 ± 3.8	81 ± 2.4	81 ± 3.4
HP				
Avg 24 hr Fe_{59} uptake*—Femur	.12 ± .03	.09 ± .02	.09 ± .02	.10 ± .01
Blood	.29 ± .08	.24 ± .04	.29 ± .02	.28 ± .02
Spleen	.08 ± .01	.14 ± .05	.19 ± .04	.18 ± .03
Avg spleen wt (g)	.10 ± .02	.14 ± .04	.15 ± .05	.16 ± .01
Avg ht	74 ± 3.8	75 ± 3.0	72 ± 3.4	73 ± 2.8
HP + PV				
Avg 24 hr Fe_{59} uptake*—Femur	1.8 ± .09	.10 ± .02	.10 ± .01	.07 ± .01
Blood	5.8 ± 1.2	22.8 ± 1.5	27.4 ± 2.8	33.0 ± 3.2
Spleen	2.8 ± .07	15.3 ± 2.1	19.8 ± 1.7	20.7 ± 1.8
Avg spleen wt (g)	.37 ± .09	.98 ± .12	2.74 ± .84	2.89 ± .54
Avg ht	76 ± 4.8	79 ± 5.4	79 ± 4.2	84 ± 2.1

* Average from minimum of 5 mice/group. ± = Standard deviation.

TABLE III. Plasma and Urine Erythropoietin Levels in Normal (N) and Hypertransfused-Polycythemic (HP) Ha/ICR Swiss Mice Infected with Polycythemic Virus (PV).

Group	Days post-infection	Ht level of infected mice (donor)	% 24 hr blood Fe_{59} uptake in HP mice	
			Plasma	Urine
(A) Infected				
N + PV	1–10	44 (39–51)	.32 (.25–.41)	.29 (.22–.35)
	11–20	56 (49–64)	.31 (.22–.40)	.29 (.29–.30)
	21–28	79 (75–84)	.25 (.18–.35)	.32 (.28–.41)
HP + PV	1–10	77 (74–82)	.24 (.21–.27)	.20 (.15–.25)
	11–20	75 (71–79)	.30 (.27–.34)	.27 (.20–.34)
	21–28	78 (69–90)	.25 (.18–.35)	.28 (.22–.32)
(B) Non-infected				
N	0	41 (40–42)	.28 (.25–.31)	.34 (.27–.39)
HP	0	75 (72–78)	.21 (.18–.25)	.29 (.24–.33)

cell proliferation accompanying a marked erythropoiesis. For some unknown reason, the bone marrow did not show any evidence of erythropoietic stimulation; if anything, erythropoiesis was depressed. Some of the effects mentioned above can be seen as early as 3 days post-infection. When the hematocrits were taken at various days post-infection, increases were noted with time: at 7 days the hematocrit was 49%, at 14 days 57%, at 21 days 81%, and at 28 days 81%. It was not unusual to see hematocrits above 90%. Concomitant increases in hemoglobin and red blood cells were also observed. When comparisons of the 24-hour Fe_{59} uptake of the femur, blood, and spleen were made, the one thing that stood out was the increased Fe_{59} uptake of the spleen. At various times post-infection the spleen uptakes were: 1st week 6.9%, 2nd week 18.6%, 3rd week 21.2% and 4th week 18.9%. The non-infected normal mice splenic Fe_{59} uptakes ranged from 1.1% to 1.9%. The reticulocyte count increased progressively at various days post-infection: at 7 days 2.2%, 14 days 2.9%, 21 days 4.1%, and 28 days 5.3%.

HP mice infected with polycythemic virus. HP mice infected with polycythemic virus displayed a progressive increase in spleen weights. Histological findings of the spleens throughout the investigation were similar to those seen in normal mice infected with the polycythemic virus. Erythropoiesis was re-established as early as 7 days after infection and at this time the Fe_{59} uptake of the femur was 1.8%, blood 5.8%, and spleen 2.8%. At 14, 21 and 28 days Fe_{59} uptake by the blood and spleen increased markedly and was, respectively, at 14 days 22.8% and 15.3%, at 21 days 27.4% and 19.8%, and at 28 days 33.0% and 20.7%. It is interesting to note that Fe_{59} uptake by the bone marrow was decreased to 0.10% at 14 and 21 days, and to 0.07% at 28 days as splenomegaly developed. Reticulocyte counts at this time were: at 7 days 2%, 14 days 3.8%, 21 days 4.2%, and 28 days 6.8%.

Examination of the spleens of these mice showed marked erythropoiesis as well as reticulum cell proliferation. The marked erythropoiesis seen at the erythropoietic foci of the spleen, particularly during the early post-infection period, was similar to what is seen when erythropoietin is given to non-infected HP mice. Proerythroblasts, erythroblasts, and mature red blood cells were evident in the areas of the red pulp.

Erythropoietin levels. Plasma and urine from normal and HP mice infected with polycythemic virus were assayed in HP mice for erythropoietin activity. With the marked erythropoiesis induced by this virus, it was felt at some stage some amount of erythropoietin could be detected. However, none was observed at any time (Table III).

Discussion. The exact mechanism by which this polycythemic virus initiates erythropoiesis in a HP state is not known. One can postulate that the polycythemic virus influences erythropoietin-producing cells to produce erythropoietin. This production of erythropoietin could be responsible for differentiating a hemopoietic precursor or stem cell to erythroid cells. Whether this is a possible mech-

anism is not likely since detectable amounts of erythropoietin activity in the plasma and urine at any time post-infection were not observed. Moreover, there is no evidence to date that a virus can influence the production and/or release of a hormone.

Another possible mechanism is that the polycythemic virus *per se* initiates erythropoiesis independently of erythropoietin. If this be so, the polycythemic virus, like erythropoietin, is capable of enzyme induction in the stem cells, causing them to differentiate into proerythroblasts(9). The fact that the polycythemic virus caused an increase in proerythroblasts, erythroblasts, and reticulocytes, an increase in Fe_{59} uptake of the spleen, blood and, initially, in the bone marrow, without detectable erythropoietin activity in the plasma and urine, implies more favorably that the polycythemic virus acts directly upon hemopoietic precursor or stem cells independently of erythropoietin. The best way to resolve which postulation is correct is through the use of an anti-erythropoietin(10); however, our attempts to solve the problem in this way have been unsuccessful to date.

Summary. Like erythropoietin, a murine polycythemic virus can initiate erythropoiesis in a hypertransfused-polycythemic state. The mechanism by which this virus is able to do this is not known.

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1. Jacobson, L. O., Goldwasser, E., Gurney, C. W., in Ciba Foundation Symposium on Haemopoiesis, London, J. & A. Churchill, Ltd., 1960, p423.

2. Gurney, C. W., Fried, W., Proc. Nat. Acad. Sci., 1965, v54, 1148.

3. Mirand, E. A., Gordon, A. S., Wenig, J., Nature, 1965, v206, 270.

4. Fried, W., Gurney, C. W., ibid., 1965, v206, 1160.

5. Mirand, E. A., Ann. N. Y. Acad. Sci., Conference on Erythropoietin, 1967, in press.

6. Friend, C., J. Exp. Med., 1957, v105, 307.

7. Mirand, E. A., Prentice, T. C., Proc. Soc. Exp. Biol. & Med., 1957, v95, 164.

8. Mirand, E. A., Gordon, A. S., J. Endocrinology, 1966, v78, 325.

9. Lajtha, L. G., Oliver, R., in Ciba Foundation Symposium on Haemopoiesis, London, J. & A. Churchill, Ltd., 1960, p289.

10. Schooley, J. C., Garcia, J. F., Proc. Soc. Exp. Biol. & Med., 1962, v109, 325.

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Sensitization of the Mouse to Bradykinin.* (32147)

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Mice inoculated with a *Bordetella pertussis* phase I vaccine develop an enhanced susceptibility to a variety of agents and conditions such as histamine(1), serotonin(2), anaphylaxis(3), endotoxin(4), peptone(5), etc. The possible interrelationship of histamine and serotonin(6) and the suggestion that a plasma kinin(7) might play a role in systemic anaphylaxis prompted the following study of the sensitivity of the pertussis-treated mouse to bradykinin.

Materials and methods. Female Carworth Farm mice (CFW) of about 20 g weight were maintained on Purina Laboratory Chow with drinking water allowed *ad lib. B. pertussis* phase I cells, 6×10^9, in 0.5 ml volume were injected intraperitoneally (i.p.) into each mouse followed on the fourth subsequent day by intravenous (i.v.) inoculation of 0.4 mg of synthetic bradykinin† dissolved in 0.5 ml of saline solution.

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