THE KINETICS OF IRON METABOLISM IN SWINE WITH VARIOUS EXPERIMENTALLY INDUCED ANEMIAS*

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(Received for publication, October 3, 1955)

In the preceding publication (1) the results of studies on the kinetics of iron metabolism (2–5) in normal growing swine were reported. The purpose of this paper is to present the results of similar studies in swine with three different types of experimentally induced anemias. As an example of a severe hemolytic process with no detectable impairment in the capacity of the bone marrow to produce erythrocytes, anemia induced by the administration of phenylhydrazine (6) has been studied. The kinetics of iron metabolism have been studied also in swine with anemia due to a deficiency of pyridoxine (6–8) and in swine with anemia due to a deficiency of pteroylglutamic acid (9–11). The last two anemias are quite different morphologically. That anemia associated with pyridoxine deficiency is microcytic and hypochromic, whereas that occurring in pteroylglutamic acid deficiency is macrocytic. In both, the plasma iron level is greatly elevated, there is a reduction in the amount of free protoporphyrin in the erythrocytes, and there is erythroid hyperplasia in the bone marrow. However, the tissues of the pyridoxine-deficient animals are heavily laden with iron, whereas hemosiderin deposits are not prominent in the tissues of swine deficient in pteroylglutamic acid.

These studies were carried out in the hope of gaining an understanding of the pathogenesis of the anemia in these conditions and also with the expectation that these anemias of known and singular etiology might serve as patterns of reference for anemias which develop in human subjects. In a later publication (12) iron kinetics will be described in copper-deficient swine. The anemia accompanying that condition is microcytic and hypochromic and the level of iron in the plasma and tissues is low.

* This investigation was supported in part by a contract (No. AT(11-1)-82) Project No. 6, between the United States Atomic Energy Commission and the University of Utah and in part by a research grant (C-2231) from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

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§ Senior Fellow of the Damon Runyon Memorial Fund for Cancer Research.

Methods

Nine swine of the Chester-White breed were used in this study. Pteroylglutamic acid deficiency was produced by feeding a 26 per cent "purified" casein diet supplemented with sulfasuxidine and crude methyl folic acid antagonist (10). The diet used for the production of pyridoxine deficiency has been described previously (6). The animals given phenylhydrazine were fed the standard 26 per cent crude casein diet with a complete vitamin and mineral supplement (13).

TABLE I

Data from Which Ferrokinetic Calculations Were Made

Group	Pig No.	Body weight	Growth rate	Plasma volume	Red cell volume	Volume packed red cells	Hemoglobin	Plasma iron
		kg.	*kg./day*	*ml./kg.*	*ml./kg.*	*ml./100 ml.*	*gm./100 ml.*	*μg./100 ml.*
Control								
Mean*		28.8	0.33	47.7 ± 8.5	30.4 ± 5.4	37.6 ± 3.0	12.7 ± 1.0	166 ± 52
Range		(8.6–97.0)	(0.10–0.64)	(33.9–60.3)	(20.0–44.9)	(34.2–47.0)	(10.7–14.4)	(95–295)
Hemolytic anemia (phenylhydrazine)	13–37	32.7	0.08	69.5	20.8	22.2	5.4	323
	13–38	26.4	0.04	44.4	18.1	26.6	7.6	89
	13–39	25.0	0.07	77.7	27.3	29.0	7.4	66
Mean		28.0	0.06	63.9	22.1	25.9	6.8	159
Pyridoxine-deficient	13–43	8.5	0.10	68.8	15.0	9.0	2.9	552
	13–44	10.9	0.00	50.5	18.8	19.0	5.9	676
	13–45	9.0	0.00	72.2	13.8	10.6	3.6	526
Mean		9.5	0.03	63.8	15.8	12.8	4.1	585
Pteroylglutamic acid–deficient	13–53	18.8	0.00	55.9	15.9	20.8	6.1	696
	13–87	13.5	0.32	50.0	14.1	21.5	7.1	470
	13–88	13.8	0.24	56.9	10.9	15.9	5.7	539
Mean		15.4	0.18	54.3	13.6	19.4	6.3	568

* ± one standard deviation.

Phenylhydrazine was administered orally to 3 of the swine in daily doses during the week prior to the ferrokinetic study. 2 gm. per day was given for the first 2 days. Thereafter, the dose was adjusted daily so that the volume of packed red cells was between 20 and 30 ml./100 ml. at the time of the ferrokinetic study.

Hematologic studies (red cell count, hemoglobin concentration, volume of packed red cells, calculation of the corpuscular constants, reticulocyte, total leukocyte, and differential leukocyte counts) were performed weekly. In addition, the volume of packed red cells and hemoglobin concentration were determined daily during the ferrokinetic study.

The techniques used in this study and the calculation of the data were the same as those described in the preceding publication (1) with the exception that the radioiron was preincubated with plasma from normal swine because of the possibility that the iron-binding protein in the animals with experimentally induced anemias might already be saturated.

Data concerning the individual pigs are given in Table I.

RESULTS

Phenylhydrazine-Induced Hemolytic Anemia.—The plasma iron level was elevated in 1 of the animals (13-37) and reduced in 2 (Table I). The ferrokinetic data are presented in Table II. In all 3 of the pigs the radioactive iron disappeared rapidly from the plasma and the plasma iron turnover rate, expressed in milligrams of plasma iron turned over per kilogram of body weight per day, was greatly increased as compared with that of normal pigs.

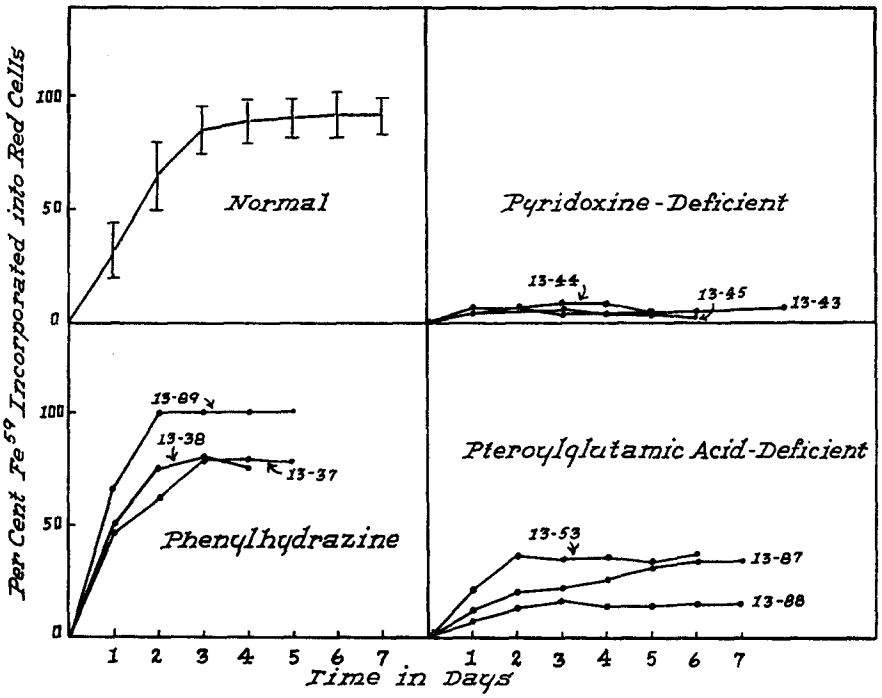

FIG. 1. Per cent of the administered dose of radioiron incorporated into the erythrocytes of normal and anemic swine. The vertical bars on the mean normal uptake curve represent one standard deviation.

The rate of incorporation of radioiron into the erythrocytes is shown in Fig. 1. All 3 of the animals incorporated the isotope into red cells more rapidly than did the normal pigs, and plateau concentrations were reached sooner. An average of 86 per cent of the administered activity was ultimately incorporated as compared with 92 per cent in the normal animals. This difference in the amount incorporated by the two groups is probably not significant since it is likely that some of the labelled red cells in the anemic animals were hemolyzed as soon as they were released into the circulation.

The average amount of iron incorporated into red cells daily by the anemic animals was 4.23 mg./kg. of body weight, a value 4.2 times the normal mean. Of this amount, 4.14 mg. or 97 per cent was turning over through the red cells since only 0.10 mg./kg. was accounted for by growth of the animals. By calculation, the average RBC life span in the animals given phenylhydrazine was 5 days as compared with a mean of 63 days in the normal pigs.

TABLE II

Ferrokinetic Data in Swine with Anemia

Group	Pig No.	T½*	PITR‡	Per cent FE^{59} incorporated into RBC	RBC IIR§	Iron incorporated due to growth	RBC ITR‖	RBC life span
		hrs.	*mg./kg. day*		*mg./kg. day*	*mg./kg. day*	*mg./kg. day*	*days*
Control								
Mean.....		1.19	1.11	92	1.01	0.42	0.59	63
Range.....		0.72–1.67	0.40–1.66	72–100	0.40–1.66	0.03–0.85	0.32–1.03	34–100
Hemolytic anemia¶	13–37	0.53	7.06	79	5.58	0.04	5.54	3
	13–38	0.22	2.98	80	3.38	0.02	2.36	7
	13–39	0.18	4.74	100	4.74	0.23	4.51	6
Mean....		0.31	4.93	86	4.23	0.10	4.14	5
Pyridoxine-deficient	13–43	1.25	5.01	6	0.30	0.10	0.20	44
	13–44	2.08	2.71	8	0.22	0.00	0.22	64
	13–45	1.13	5.55	5	0.28	0.00	0.28	38
Mean....		1.49	4.42	6	0.27	0.03	0.23	49
P.G.A.-**deficient	13–53	1.10	5.97	36	2.15	0.00	2.15	8
	13–87	0.70	5.58	35	1.95	0.36	1.59	11
	13–88	1.03	4.93	14	0.69	0.22	0.47	31
Mean....		0.94	5.49	28	1.60	0.19	1.40	17

* The time at which the concentration of Fe^{59} in the plasma had decreased to half of its initial value.

‡ Plasma iron turnover rate.

§ Red cell iron incorporation rate.

‖ Red cell iron turnover rate.

¶ Due to the ingestion of phenylhydrazine.

** Pteroylglutamic acid.

Anemia Due to Pyridoxine Deficiency.—The plasma iron level was elevated in all 3 animals (Table I). The ferrokinetic data are presented in Table II. In 2 of the pigs the radioiron disappeared from the plasma at the normal rate. The disappearance rate in the 3rd animal (13-44) was slower than normal. The plasma iron turnover rate was greatly increased in all 3 pigs. The rate of incorporation of iron into erythrocytes was slower than in normal

animals and an average of 6 per cent of the administered radioactivity was ultimately incorporated into the red cells as compared with 92 per cent in the normal pigs. The average amount of iron incorporated into erythrocytes each day was 0.27 mg./kg. of body weight or only about one-fourth of the average amount incorporated into the erythrocytes of the normal swine. The average life span of the erythrocytes was calculated to be 49 days, a value within the normal limits.

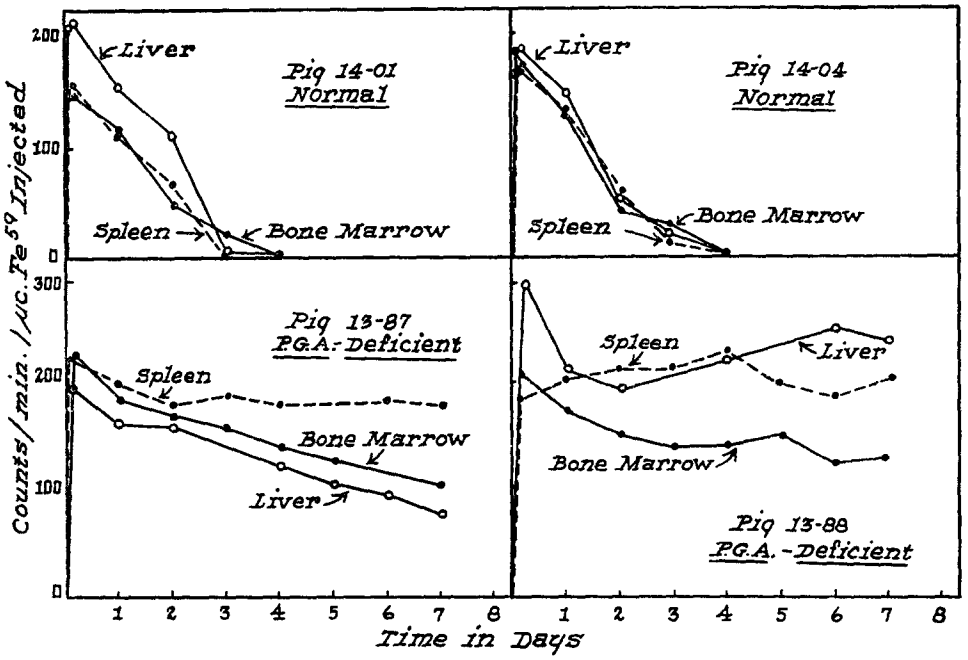

FIG. 2. Body surface radioactivity in 2 normal pigs and in 2 pigs deficient in pteroylglutamic acid (P.G.A.).

Anemia Due to a Deficiency of Pteroylglutamic Acid.—The plasma iron level was elevated in all 3 animals (Table I) and the radioactivity disappeared from the plasma at a normal rate in 2 pigs and at a slightly reduced rate in 1 animal (13-87). The plasma iron turnover rate was about 5 times the normal mean in all 3 of the pigs (Table II). The rate of incorporation of iron into erythrocytes was slower than in normal animals and an average of only 28 per cent of the administered isotope was incorporated into the red cells. However, the amount of iron incorporated into red cells each day was increased beyond the upper limit of normal in 2 pigs and was within the normal range in 1 of them. After adjustment for the growth of the animals, the amount of iron turned over through the red cells each day was found to be

increased in 2 and normal in 1 pig (13-88). The average amount of iron turned over through the red cells by the 3 pigs was approximately twice normal. The calculated red cell life span was shortened in all three animals. The mean value was 17 days as compared with 63 days in the normal pigs.

Body surface radioactivity was determined over the liver, spleen, and sacral bone marrow sites of two of the pteroylglutamic acid-deficient pigs. The results are presented in Fig. 2 and are compared with the results obtained in 2 normal swine. No significant difference was noted in the rate of uptake of radioiron into the three sites as compared with that in normal animals. Although the height of the initial peak was somewhat higher in the deficient animals than in the normal ones, this difference is probably not significant since the deficient animals had less subcutaneous tissue and the counter was closer to the target organ. The rate of release of the isotope from the liver, spleen, and sacral bone marrow sites of the deficient animals was greatly retarded.

DISCUSSION

In the previous publication (1) the assumptions and errors involved in ferrokinetic studies were discussed. Ferrokinetic calculations are made on the assumption that the rate of production of cells is equal to the rate of their destruction, and therefore, that red cell volume is constant. To maintain anemic animals in a state of hemopoietic equilibrium for any period of time is indeed difficult. On the other hand, the deficient animals and the animals given phenylhydrazine grew very little, if at all, during the period of study. In order to make the data comparable to those in rapidly growing control animals we have corrected for the small degree of growth of the anemic pigs although this correction does not modify the data significantly.

The ferrokinetic studies revealed striking differences between the 3 experimentally induced anemias. In the animals given phenylhydrazine the life span of the erythrocytes was shortened markedly. The values obtained for the life span in these animals are only approximate because it was difficult to maintain a constant volume of packed red cells during the period of study. Furthermore, when the life span of the erythrocytes is shortened so drastically it is difficult to determine the exact proportion of isotope which is incorporated into the red cells since a portion of the cells is destroyed as rapidly as they are labelled and discharged into the blood (2). Finally, the determination of the red cell volume with P^{32}-tagged red cells may not be accurate under such conditions since some of the tagged cells may be rapidly eliminated with the result that the value may be falsely high.

Animals given phenylhydrazine incorporated 4.2 times as much iron into hemoglobin per day as did the normal pigs. Since this rate of production was not sufficient to prevent the development of anemia it is likely that this was the maximum rate at which the bone marrows of these animals could produce

hemoglobin. If one assumes that 100 per cent of the iron turned over through the plasma actually entered the bone marrow for use in erythropoiesis, the red cell output, as measured by the red blood cell iron incorporation rate, increased fivefold. If it is assumed that the phenylhydrazine did not impair the capacity of the marrow to produce erythrocytes, then the bone marrow of normal swine can increase the output of red cells by a factor of four to five. This value is similar to, but a little less than, that estimated in man (14). This means that if the capacity of the marrow to produce cells is not impaired, the marrow can compensate for a reduction in the life span of the cells to one-fourth or one-fifth of the normal value. Since the life span of porcine erythrocytes is normally about 63 days (1, 13), a normal marrow should be able to produce enough erythrocytes to prevent the development of anemia if the life span is not shorter than 13 to 16 days.

The ferrokinetic pattern in the pyridoxine-deficient swine was quite different from that in the animals given phenylhydrazine. In this deficiency the life span of the cells was within normal limits, and only 0.27 mg. of iron per kg. of body weight per day was incorporated into hemoglobin. Therefore, these animals were producing about one-fourth as much hemoglobin as the normal animals and about one-sixteenth as much as the animals given phenylhydrazine. Thus, the anemia in this deficiency was due to an inability of the marrow to produce a normal number of cells.

The high plasma iron turnover rate in pyridoxine deficiency is interesting in view of the fact that only about 0.4 per cent of the iron which was turned over each day through the plasma appeared in the erythrocytes. Two explanations for this phenomenon come to mind. It is known that the tissue stores of iron are greatly increased in pyridoxine deficiency (7) and it is possible that there is a rapid turnover between the plasma and the tissue iron. The other explanation is that the iron goes to the marrow, is incorporated into heme, and then the heme is catabolized before it can appear in the cells in the circulation. That this is an unlikely explanation is indicated by the observation that the excretion of bile pigment is not increased (6).

Pyridoxine deficiency in swine differs from aplastic anemia in human subjects in that in the latter condition the plasma iron turnover rate is normal or decreased (2, 5). It does, however, from a kinetic standpoint, resemble the refractory pancytopenia associated with a cellular marrow (5).

The mean survival time of the erythrocytes of the animals deficient in pteroylglutamic acid was decreased to an average of 17 days. However, the bone marrow was able to increase its production rate only about 1.6 times the normal rate. Thus, anemia developed because of two defects: a decrease of the erythrocyte survival time and a limitation of the capacity of the bone marrow to increase the production of cells to the same extent as in the animals given phenylhydrazine.

The similarities between the ferrokinetic patterns in pteroylglutamic acid-

deficient swine and patients with pernicious anemia in relapse are striking. In both conditions the plasma iron level is increased (6, 8, 15), the amount of iron turned over through the plasma each day is increased (2, 16, 17), the red cell uptake of radioiron is low (2), the red cell iron turnover rate is normal or increased only slightly (2), and the survival time of the erythrocytes is shortened (18–20). It has been suggested by several observers (5, 16, 21, 22) that there may be rapid destruction of red cell precursors in the marrow of patients with pernicious anemia. If such is the case, there would be an iron compartment in the marrow with a short turnover time through which recycling of iron would take place. This would account for the excessive turnover of iron through the plasma. Our data in the pteroylglutamic acid–deficient swine could also be interpreted in this manner. If this interpretation is correct, it would be expected that the excretion of bile pigment would be increased as it is known to be in pernicious anemia. Unfortunately, such measurements have not been made in pteroylglutamic acid–deficient swine.

Another explanation for the fivefold increase in the plasma iron turnover rate in the presence of a mean red cell iron incorporation of only 1.6 times normal is that there is considerable exchange of iron between the non-hemoglobin tissues and the plasma. The body surface counts do not help to clarify this problem since the delivery of isotope from the bone marrow was retarded to the same degree as that from the liver and spleen. Furthermore, it is possible that much of the activity attributed to the liver and spleen was due to the proximity of the ribs to these sites (1).

In the past, the anemias of nutritional origin have been classified as being due to decreased red cell production (23, 24). The data presented in this paper suggest that the pathogenesis of all types of anemia due to nutritional deficiency may not be similar. A shortened erythrocyte life span has now been demonstrated not only in anemia due to pteroylglutamic acid deficiency in swine but also in swine deficient in copper (12), in scorbutic human subjects with anemia (25, 26), and in patients with pernicious anemia (18–20). Thus, it is apparent that decreased blood production is not the only factor involved in the pathogenesis of some anemias of nutritional origin, although this is apparently the only factor involved in the pathogenesis of the anemia due to a deficiency of pyridoxine in swine. Current knowledge concerning the kinetic pattern in various forms of nutritional anemia in man and swine is summarized in Table III.

The mechanisms whereby the life span of erythrocytes is decreased in the different deficiency states have not been adequately studied. Those studies which have been done suggest that the mechanism may not be the same in each deficiency. Studies in this laboratory (12) on the nature of the hemolytic mechanism of the anemia of copper deficiency in swine suggest that the defect is primarily intracorpuscular. Studies by the Ashby technique

indicate that normal red cells are destroyed at an accelerated rate in scorbutic individuals (25). Data are not available on the survival of cells from patients with scurvy transfused into normal subjects. Several observers (18, 19) have demonstrated that the life span of cells from a patient with pernicious anemia, transfused into a normal recipient, is moderately impaired. It has been suggested that, in addition to the intracorpuscular defect in this disease, there is an extracorpuscular hemolytic mechanism (27).

Several general observations can be made from the ferrokinetic data obtained in this study. The PITR was increased to approximately the same extent in each experimental group and yet red cell production ranged from markedly reduced to markedly increased. Thus, it is apparent that the plasma iron turnover rate is of no value in the estimation of the rate of erythropoiesis. The uptake of Fe^{59} into the red cells was markedly reduced in pteroylglutamic acid deficiency and yet the production of red cells was apparently greater than in the normal animals. Thus, the uptake of radioiron by itself is of no value in predicting the rate of red cell synthesis. A complete study must be performed in order to understand the kinetic pattern.

TABLE III

Kinetic Pattern in Nutritional Anemia

Deficiency	Plasma iron level	Plasma iron turnover rate	RBC iron turnover rate	Erythrocyte survival time
Pyridoxine (swine)	Increased	Increased	Decreased	Normal
Copper (swine)	Decreased	Increased	Increased slightly	Decreased
Pteroylglutamic acid (swine)	Increased	Increased	Normal or slightly increased	Decreased
Vitamin B_{12} (human subjects)	Increased	Increased	Normal or slightly increased	Decreased
Ascorbic acid (human subjects)	Normal			Decreased

SUMMARY

Ferrokinetic studies were performed on 3 swine given phenylhydrazine, 3 swine deficient in pyridoxine, and 3 swine deficient in pteroylglutamic acid. Body surface radioactivity was measured in 2 pteroylglutamic acid–deficient animals.

In the animals given phenylhydrazine, the mean erythrocyte survival time was 5 days. The plasma iron turnover rate was increased about fourfold, and the rate of erythropoiesis was four to five times greater than that in the control pigs.

In the pyridoxine-deficient swine, the mean erythrocyte survival time was within the limits of normal. The plasma iron turnover rate was increased

fourfold, but the rate of erythropoiesis was approximately one-fourth the normal mean value. These data are interpreted as indicating that the anemia associated with this deficiency is a result of an inability of the bone marrow to produce a normal number of erythrocytes.

In the pteroylglutamic acid–deficient swine, the mean erythrocyte survival time was 17 days. The plasma iron turnover rate was 5 times the normal mean value. The rate of erythropoiesis was 1.6 times greater than the mean value in the control pigs. These data are interpreted as indicating that anemia develops in this deficiency as a result of a combination of a shortening of the erythrocyte survival time and a limitation of the capacity of the bone marrow to increase red cell production to the same degree as a normal marrow.

The radioactivity in the liver, spleen, and bone marrow of the pteroylglutamic acid–deficient swine, as determined by measurement of the radioactivity over the body surface, declined more slowly than in control pigs.

The authors are indebted to Dr. A. Gibson, Merck and Company, Inc., Rahway, New Jersey, for the supplies of thiamin hydrochloride, riboflavin, nicotinic acid, pyridoxine hydrochloride, calcium pantothenate, inositol, *para*-amino-benzoic acid, choline chloride, and cobalamin; to Dr. T. H. Jukes, Lederle Laboratories, Pearl River, New York, for the pteroylglutamic acid; to Dr. E. L. Severinghaus, Hoffman-La Roche, Inc., Nutley, New Jersey, for biotin and vitamins A, D, E, and K.

Misses Doris Kurth and Mitsue Yanagita, and Messrs. George Trappett and Ocie Hadley gave valuable technical assistance.

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