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Research Article

THE ANEMIA OF THERMAL INJURY. III. ERYTHROPOIESIS AND HEMOGLOBIN METABOLISM STUDIED WITH N^{15}-GLYCINE IN DOG AND MAN [1]

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In earlier investigations regarding the anemia of thermal injury (1) tentative conclusions were reached suggesting that the deficiency in total circulating hemoglobin was produced by at least two mechanisms: In the first few days after a burn, a hemolytic process; then, later, inadequate hemoglobin synthesis. To explore further the dyshematopoiesis, experimental studies have been performed in burned dogs and in a burned man. The principal index of red cell and hemoglobin formation was the rate and extent of uptake of heavy nitrogen (N^{15}) from isotopically-labelled glycine into the heme fraction of hemoglobin. This technique also makes it possible to estimate the life span of the erythrocytes formed during the particular phase of injury under study (2). Numerous additional physiological and biochemical studies were performed concomitantly to aid in the interpretation of the N^{15} data. These included complete hemograms, marrow surveys on the dogs, measurements of blood volume, nitrogen balance, serum iron, copper and bilirubin, erythrocyte protoporphyrin, urinary coproporphyrin, and urinary and fecal urobilinogen.

METHODS AND MATERIALS

Mongrel male dogs, two to four years of age, were kept in metabolic cages on a fixed diet designed to give a daily intake of 14 Gm. of nitrogen and sufficient calories to maintain body weight under control conditions. Hemoglobin was measured by the alkaline hematin method in sodium carbonate on the Coleman Junior Spectrophotometer standardized for hemoglobin by the oxygen capacity method. Reticulocytes were counted on a wet preparation after staining with brilliant cresyl blue (Coleman and Bell Co.). Hematocrits and cell counts were determined by standard procedures as given by Wintrobe (3). Blood volume was measured with T-1824 dye (4).

Feces, collected in four-day periods, were homogenized and made to a constant volume from which aliquots were analyzed for nitrogen (5) and urobilinogen (6). Urine collections were analyzed daily for nitrogen, urobilinogen, and coproporphyrin (7). Serum iron (8), copper (9), bilirubin (10), and erythrocyte protoporphyrin (11) were determined at intervals of about ten days.

When nitrogen equilibrium was attained, the experimental animals were given a standard contact-burn of 20 per cent body surface area (12). Approximately ten days after burning, at the height of the catabolic response and when anemia was developing, they were fed 10 Gm. of glycine containing 33.8 atom per cent excess N^{15} in 2.0 Gm. amounts every two hours for five doses.[6] In the periods immediately after glycine feeding, urine collections were fractionated at eight-hour intervals for study of N^{15} excretion in urinary nitrogen. Hemin was isolated as described by Shemin and Rittenberg (13) and recrystallized according to Fischer (14), then analyzed in duplicate for N^{15} content in the mass spectrometer. Stercobilin was isolated by a method modified slightly from that described by Watson (15), recrystallized from chloroform and dried over P_2O_5. Purity was checked by optical rotation on each sample sent for isotope analysis. Quantity was usually sufficient for analysis in duplicate. All samples for N^{15} analysis were routinely compared with tank nitrogen as a reference standard, and the N^{15} atom per cent excess over tank nitrogen determined. A table for calculation of N^{15} concentration was computed and has been found very useful in this work (16). The Nier-type isotope-ratio mass spectrometer used in these and subsequent experiments was built and maintained by Dr. William T. Ham, Jr., Mr. Ray Williams, and Mr. Fred Schmidt.

Dog 29 served as his own control. The experimental

[1] This study was conducted under Contract Number DA 49-007-MD-99 between the Office of the Surgeon General, Department of the Army, Washington, D. C., and the Medical College of Virginia, Richmond, Virginia.

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[6] Dog 17 was given glycine containing 31.6 atom per cent excess N^{15}.

burn was produced, and studies were done. When he had apparently achieved full recovery, the glycine feeding was repeated. The reverse procedure was planned for the second control, dog 31, but he died unexpectedly before the burn was given.

RESULTS AND DISCUSSION

Animal experiments

The uptake of N^{15} into the pigment fraction of the hemoglobin molecule, as measured by the atom per cent excess in the isolated hemin, needs both a quantitative and kinetic interpretation. Since tagged heme is always diluted by pre-existing untagged hemoglobin, the total quantity of the latter will in part determine, by dilution alone, the concentration of the isotope in the isolated hemin. (The terms hemoglobin, heme, and hemin may be used interchangeably in this discussion so far as N^{15} uptake is concerned.) Table I presents data comparing the total quantity of N^{15} incorporated into the heme of the experimental animals. The uptake is expressed both in milligrams of N^{15} and as a percentage of the total N^{15} administered. The data clearly show that although the difference in atom per cent excess N^{15} between control dogs 31 and 29 was greater than 100 per cent, due to the difference in amount of circulating hemoglobin, the total N^{15} uptakes expressed in milligrams (1.89 and 1.49) and as per cent of administered N^{15} (0.28 and 0.22 per cent) are not very different. Calculated in the same manner, the quantities incorporated in the burned dogs 29, 16, and 17 were one-sixth to less than one-twentieth of those in the normal animals. Because of the somewhat lower body weight of dogs 16 and 17 (Table I), the amount of N^{15} received per kilogram of body weight was actually slightly more in these animals (Table III).

A decrease in total circulating hemoglobin (T.C.H.) is usually one important stimulator of increased erythropoiesis. In all three burned animals, T.C.H. was diminishing at the time of glycine feeding as can be seen best by reference to Figures 1 and 2. On the day of glycine feeding when maximum N^{15}-glycine concentrations were present for heme formation, reductions in T.C.H. of 16 to 40 per cent had occurred (Table II). Hemin N^{15} data suggest that hemoglobin formation at the tenth day post-burn was significantly depressed even in the presence of a falling total circulating hemoglobin, a known stimulus to erythropoiesis. In the next 20 days reduction in T.C.H. became as much as 27 to 51 per cent, and reticulocytosis became evident, but at this time healing of the burned areas was well under way. Obviously the results which are obtained will depend on what time after the thermal trauma the glycine is fed. The ten day period was chosen because studies (12) have demonstrated maximum anemia at that time. The hemolytic phase of burn anemia was considered to be over at this time since the animals did not show hyperbilirubinemia.

The rates of uptake of N^{15} into heme in the control animals were quite similar, as seen in the composite graph Figure 3. But, in the burned dogs, when compared with the controls, distinct differences in uptake rates are at once evident. The data on dog 29 (Figure 1) are particularly strik-

TABLE I

Comparison of N^{15} uptake in the control and the burned animals

Dog No.	Condition	Wt. Kg.	Average total circulating heme over period of glycine uptake mg.		N^{15} in heme at 25 days post-burn; 15 days after glycine feeding: atom per cent excess*	mg.	Per cent of administered N^{15}
31	Control	18.1	8,966	(247)†	0.218	1.89	0.28
29	Control	20.0	15,319	(422)	0.100	1.49	0.22
29	Burn	20.0	10,055	(277)	0.024	0.24	0.03
16	Burn	16.3	7,042	(194)	0.012	0.08	0.01
17	Burn	16.3	6,098	(168)	0.041	0.24	0.04

* These values have been corrected for the natural abundance of N^{15} in normal hemin which we found to be 0.004 to 0.005 atom per cent higher than tank nitrogen. This increased abundance of N^{15} is of the same magnitude found in other compounds of biological origin, *e.g.*, urinary urea, ammonia, and uric acid (35).

† Figures in parentheses are the average total circulating hemoglobin (Gm.) over period of glycine uptake calculated from 2 or 3 T.C.H. determinations 10 to 31 days after burn (1.0 Gm. hemoglobin equivalent to 36.3 mg. heme).

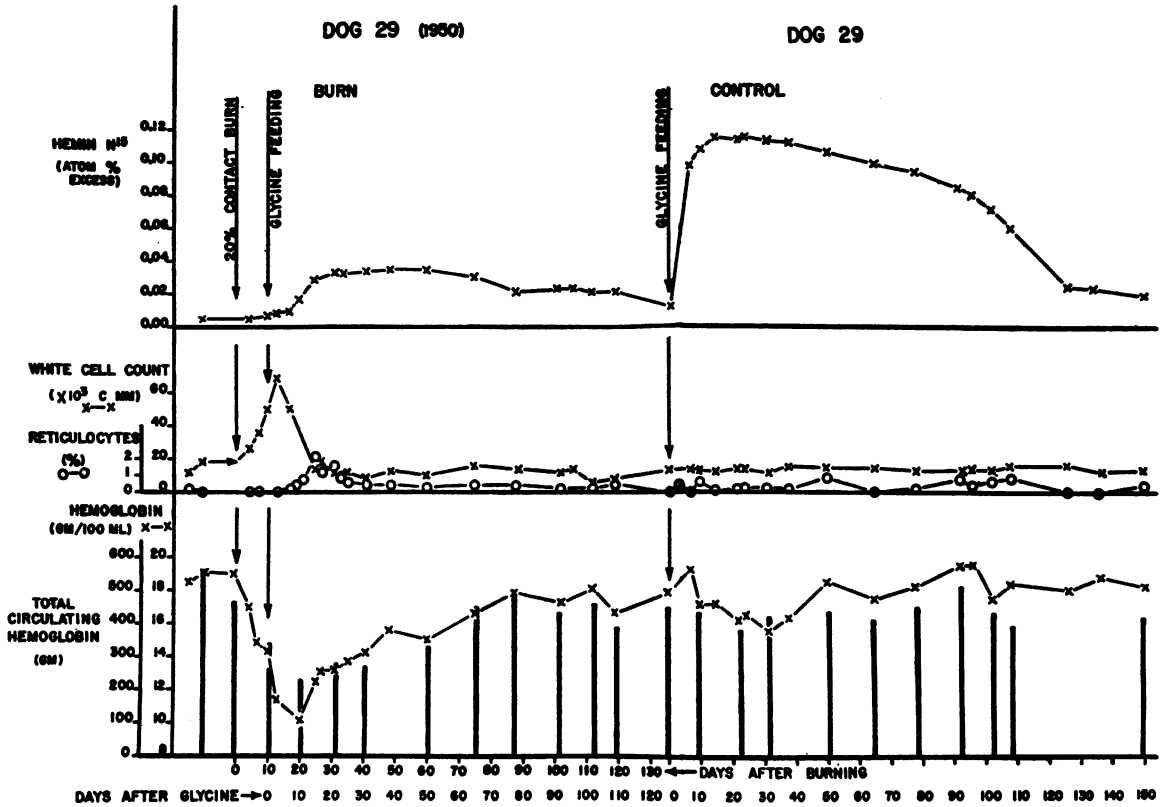

FIG. 1. INCORPORATION OF HEAVY NITROGEN FROM N^{15}-GLYCINE INTO HEME OF A BURNED DOG WHICH SERVED AS HIS OWN CONTROL: CORRELATION WITH CHANGES IN TOTAL CIRCULATING HEMOGLOBIN, LEUCOCYTES, AND RETICULOCYTES

ing since this animal served as his own control. In the normal state almost maximal uptake of glycine had been obtained 10 days after the feeding. After the same length of time, but when fed 10 days after a 20 per cent contact burn, only a slight incorporation of glycine had occurred. An increased rate of tagging appeared coincident with a beginning reticulocytosis after the 20th day post-burn. From this time on a steady gain in T.C.H. was evident, indicating the inhibition accompanying injury was no longer depressing erythropoiesis. Dogs 16 and 17 (Figure 3) which succumbed after 49 and 35 days, respectively, again demonstrate an overall diminished rate of synthesis as compared to the controls. In dog 16 (Figure 2) there was a slight incorporation of N^{15} into heme from the 10th to the 25th day post-burn after which, preceded by a slight reticulocytosis of 2 per cent,

TABLE II

Change in total circulating hemoglobin at the day of glycine feeding and during the time of maximum N^{15} incorporation into newly formed hemoglobin

Dog No.	Condition	Control T.C.H. *Gm.*	T.C.H. at day of glycine feeding *Gm.*	Per cent change %	T.C.H. (ave.) 10–31 days post-burn *Gm.*	Per cent change %
31	Control	263	223	−15.2	247	− 6.0
29	Control	423	456	+ 7.8	422	0.0
29	Burn	567	337	−40.5	277	−50.9
16	Burn	269	226	−16.0	194	−27.9
17	Burn	297	221	−25.6	168	−43.4

TABLE III

Total urinary and fecal N^{15} excretion in the first 96 hours after feeding

Dog No.	Condition	Total N^{15} administered		N^{15} excreted		Per cent excreted		Total N^{15} excreted at 10 days
		mg.	*mg./Kg.*	Urine *mg.*	Feces *mg.*	Urine %	Feces %	%
31	Control	675	37	356	5.1	52.7	0.76	61.3
29	Burn	675	34	275	5.7	40.7	0.84	45.8
16	Burn	675	41	426	18.0	63.1	2.67	74.0
17	Burn	622	38	489	10.7	78.6	1.72	90.0

a more marked rise in N^{15}-hemin level became evident. Dog 17 (Figure 2) showed an early sharp increase in the first four days after glycine feeding. A reticulocyte count of 0.8 per cent on the day of feeding was higher than the counts of 0.4 per cent and 0.0 per cent observed in the other burned animals, but unless it went higher in the next two days (no counts were done) this early increase in N^{15} level in this case is without explanation. Another distinct, and perhaps significant, difference between dog 17 and dogs 29 and 16 was the absence of a pronounced leucocytosis at the time of glycine feeding. From the 14th to the 30th day uptake was at a rate comparable to the other burned animals, despite a greater degree of reticulocytosis from the 25th to the 35th day. Terminally, the hemoglobin concentration in this animal fell to very low levels. Autopsy disclosed numerous bleeding areas in the gastrointestinal mucosa. The late reticulocytosis thus may have been stimulated by hemorrhage.

The labelling which accompanies reticulocytosis at a time when a major portion of the N^{15} has been excreted is of particular interest. The heavy nitrogen for labelling would have to come from an endogenous "dynamic" source, since 10 to 15 days

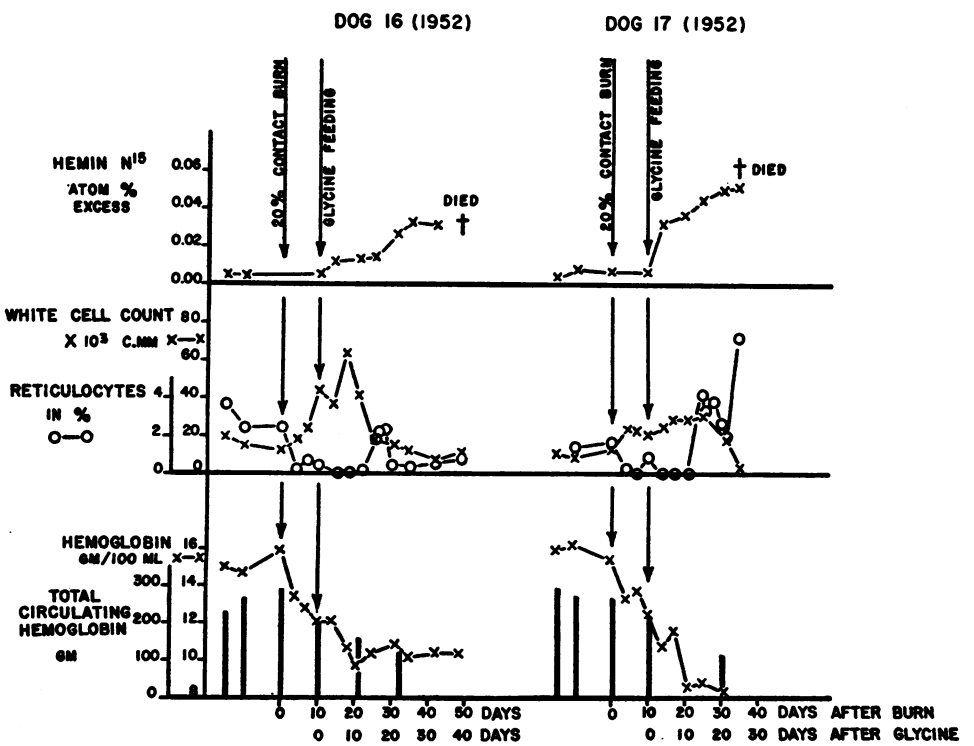

FIG. 2. INCORPORATION OF HEAVY NITROGEN FROM N^{15}-GLYCINE INTO HEME IN TWO BURNED DOGS: CORRELATION WITH CHANGES IN HEMOGLOBIN, LEUCOCYTES, AND RETICULOCYTES

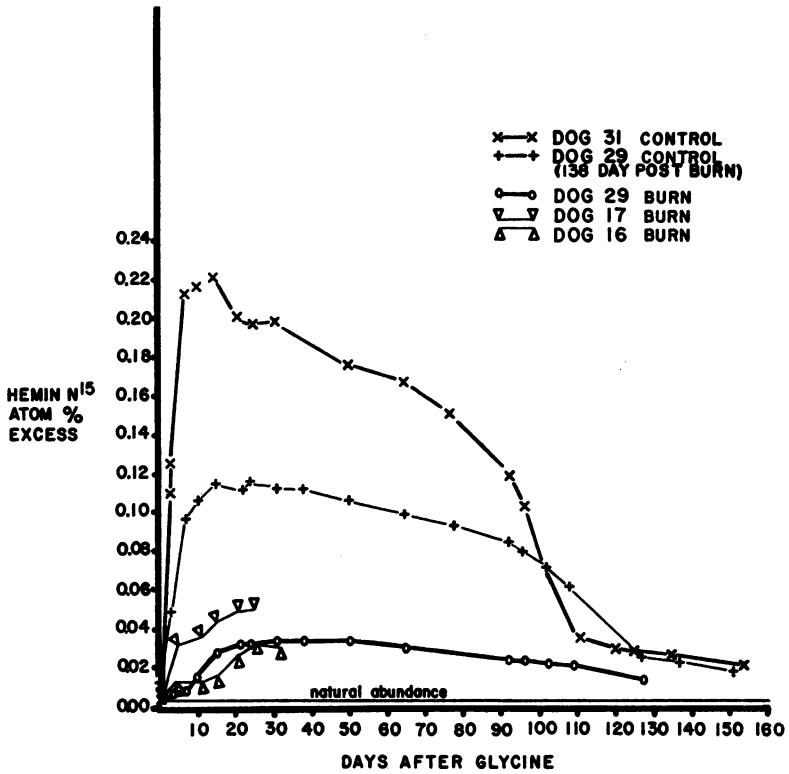

FIG. 3. COMPARISON OF THE SLOWER RATE AND SMALLER EXTENT OF N^{15} INCORPORATION INTO HEME FROM N^{15}-GLYCINE IN BURNED AND NORMAL DOGS

after the oral administration of the N^{15}-glycine the major portion of the isotope has been excreted. This source is no doubt glycine, but it is glycine that has been incorporated into the tissue and plasma proteins. It is consistent with the thesis of Whipple (17) that the dog will utilize his own tissue to form hemoglobin. A similar utilization of body nitrogen is found during the reticulocytosis stimulated by vitamin B_{12} in pernicious anemia patients (18), and a much greater than normal incorporation of N^{15} from N^{15}-glycine into heme has been noted at the time of therapeutically-induced reticulocytosis in this disease (19).

In the burned animal the early failure of uptake in hemin might have been due to (*a*) depressed uptake in marrow (failure of utilization), (*b*) failure of absorption, or (*c*) excessive elimination of heavy nitrogen in the urine and stools. In the first four days after feeding, all the burned dogs showed slightly greater fecal excretion of N^{15} than the control animals, but there was no indication of failure of absorption (Table III). In the same table data pertinent to urinary N^{15} excretion are given. During the first four days, control dog 31 lost 52.7 per cent of the administered N^{15}, burned dog 29 lost 40.7 per cent, and dogs 16 and 17 lost 63.1 per cent and 78.6 per cent, respectively. At the end of 10 days even more N^{15} had been excreted. Dogs 16 and 17 lost 74 per cent and 90 per cent respectively, while burned dog 29 had lost only 45.8 per cent and the control dog 31 had lost a total of 61.3 per cent in the urine. Thus two burned animals showed moderately increased urinary excretion while one was less than the control. These collective data are interpreted to indicate almost complete absorption of the orally administered glycine, and on a comparative basis suggest that failure of utilization rather than increased excretion was the significant factor in the decreased incorporation into heme. Studies presented (20) using radioactive iron indicated a depressed marrow activity in severe burns. The greater increased urinary excretion in dogs 16 and 17 is part of the increased catabolism present con-

tinuously from the time of the burn until death. Both of these animals had lost over 50 per cent of their body weight at the time of death.

The life span of the red blood cell in the two control animals has been estimated from the hemin curves by measuring the time in days from the mid-point in the uptake curve to the mid-point of the disappearance curve (2). Dog 31 had an average erythrocyte life span of about 100 days, and control dog 29, 104 days (Figure 3). The average life span of erythrocytes in the normal dog by the N^{15}-hemin technique has not been reported previously. The life span of the dog's erythrocytes using 1-C^{14}-glycine which labels globin, has been estimated to be 75 ± five days and 95 ± five days using two phlebotomized dogs (21). Hemoglobin was labelled in the globin fraction with lysine containing C^{14} in the epsilon carbon, and the erythrocyte life span estimated in this manner was 115 days, using a dog made anemic and hypoproteinemic by phlebotomy and low protein diet (22). Because of the death of dogs 16 and 17, and the low tag obtained in dog 29, it was not possible to estimate with certainty what the life span of red cells formed during injury might be.

The results of determinations of serum iron, copper, and free erythrocyte protoporphyrin are found in Table IV. In one of the burned dogs (dog 29) the serum iron was slightly depressed after the thermal injury and remained low for nearly 100 days, even after the total circulating hemoglobin had risen to normal. In this animal serum copper did not become elevated. In inflammation, hypercupremia was not observed in dogs (23), but it does occur in both man (24) and the rat (25). In the two other burned animals the serum iron did not fall to the expected low levels, nor was there any rise in serum bilirubin to explain the normal serum iron levels. The values for serum copper were erratic and no conclusions could be drawn. In none of the animals, either

TABLE IV

Serum iron (Fe), serum copper (Cu), and free erythrocyte protoporphyrin (EP) in control and burned dogs

Days after burn	Controls						Burns								
	Dog 31			Dog 29			Dog 29			Dog 16			Dog 17		
	EP μg/100 ml. RBC	Cu μg %	Fe μg %	EP μg/100 ml. RBC	Cu μg %	Fe μg %	EP μg/100 ml. RBC	Cu μg %	Fe μg %	EP μg/100 ml. RBC	Cu μg %	Fe μg %	EP μg/100 ml. RBC	Cu μg %	Fe μg %
−15	37	72	54							44	78	86	28	29	118
−11	44	102	43								65	95		65	118
− 8	38	119	45				57	76	103						
− 4							41	47	147						
0										39	101	69	44	78	146
10	31	108	110				33	80	103	27	106	105	29	50	108
20	30	80	50				39	89	57	13	128	87	24	106	127
31	32	80	57				39	71	79	35	67	84	36	36	103
41							34	76	63	33	75	111			
48	46	64	166				38	55	79	33	92	127			
60							34	82	64						
75	42		132				43	116	69						
88							44	75	49						
102	30	73	83				37	114	68						
106							41	71	64						
112	35	68	116				38	109	102						
136	35	94	93				37	119	175						
146				34	45	163									
158	38	87	105	36	51	116									
167				35	59	177									
186				25	65	98									
214				36	136	123									
228				43	87	194									
238				35	116	126									
244				42	97	155									
272				34	54	109									
286				34	71	100									

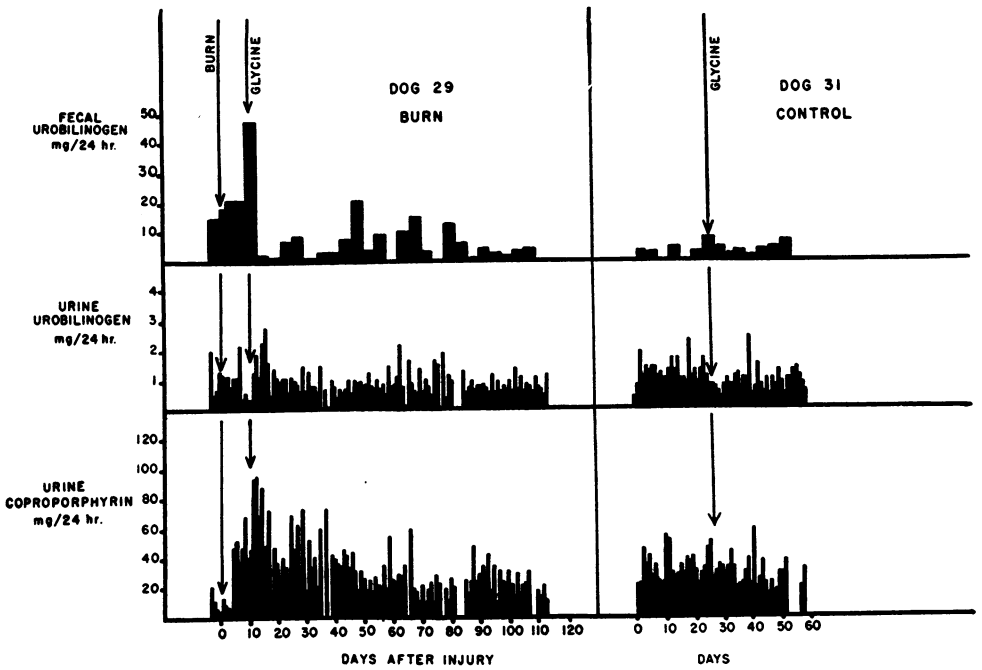

FIG. 4. FECAL UROBILINOGEN, URINARY UROBILINOGEN, AND URINARY COPROPORPHYRIN AFTER THERMAL INJURY IN THE DOG COMPARED WITH A NORMAL ANIMAL

control or burned, did we observe any rise in the free erythrocyte protoporphyrin.

The failure of the serum iron to fall markedly in the burned dogs was somewhat unexpected. A very marked and sudden diminution was found (26) following the production of staphylococcic or turpentine abscess in dogs, but a depressed serum iron after injection of staphylococcus toxin was not found, nor was production of an anemia. Anemia developed in our animals, but the serum iron did not drop. This was in striking contrast to similar studies to be described later in the burned man where there were significant changes in serum iron, copper, and free erythrocyte protoporphyrin (*vide infra*).

Figure 4 (dog 29) shows the same changes in fecal urobilinogen excretion and urinary coproporphyrin excretion which have been described in man following burns (1). From the time of the injury to the 10th day there was an increased fecal urobilinogen output. At the same time changes in urinary urobilinogen excretion were not evident, but there was a very marked increase in the urinary coproporphyrin excretion. The coproporphyrinuria began to increase on the 5th day after the burn and reached its maximum on the 12th day, two days after the glycine had been administered. No changes were observed in the control animal, dog 31. For reasons beyond our control the urinary and fecal urobilinogen and urinary coproporphyrin excretions could not be determined in the other two burned animals.

Increase in fecal urobilinogen has been attributed to the increased destruction of heme containing proteins incident to the burn. The method of determination measures both open chain tetrapyrroles (urobilinogens) from hemoglobin destruction as well as from other sources. It appears from Figure 4 (dog 29) that the glycine was administered at a time when pigment destruction was greatest. With the usual lag in the passage of intestinal contents, it is our belief that the glycine was given at a time when the major effect of hemolytic mechanisms to produce anemia had passed.

We have no certain explanation for the rise in the urinary coproporphyrin. Isomer analysis for Type I and Type III was not done. Increased urinary coproporphyrin excretion may in some manner be related to depression of erythropoiesis (24) or to liver damage (27, 28).

Human study

The clinical study was performed on a 34 year old Negro male who was burned when gasoline on his clothing became ignited. He suffered an approximate 20 per cent total burn involving right arm, chest, abdomen, and left hand, of which about 16 per cent was third degree. The past history was negative for any serious illness or operations, and he had been in good health at the time of the injury. Shortly after admission he was given tetanus antitoxin, penicillin, and in the first 24 hours 1,500 ml. of 6 per cent dextran (Macrodex). Closed dressing was applied. Ten days after admission he was partially debrided and redressed. The amount of third degree burn remaining was estimated to be about 15 to 16 per cent. The next day (eleven days after injury) oral feeding of N^{15}-glycine (31.6 atom per cent excess) was begun. He was fed a total of 24 Gm. in 2.0 Gm. rations with about 100 ml. water every four hours for twelve doses. The feeding was well tolerated and carried out without mishap. Fifteen days after the burn he complained of epigastric pain penetrating straight through to his back which

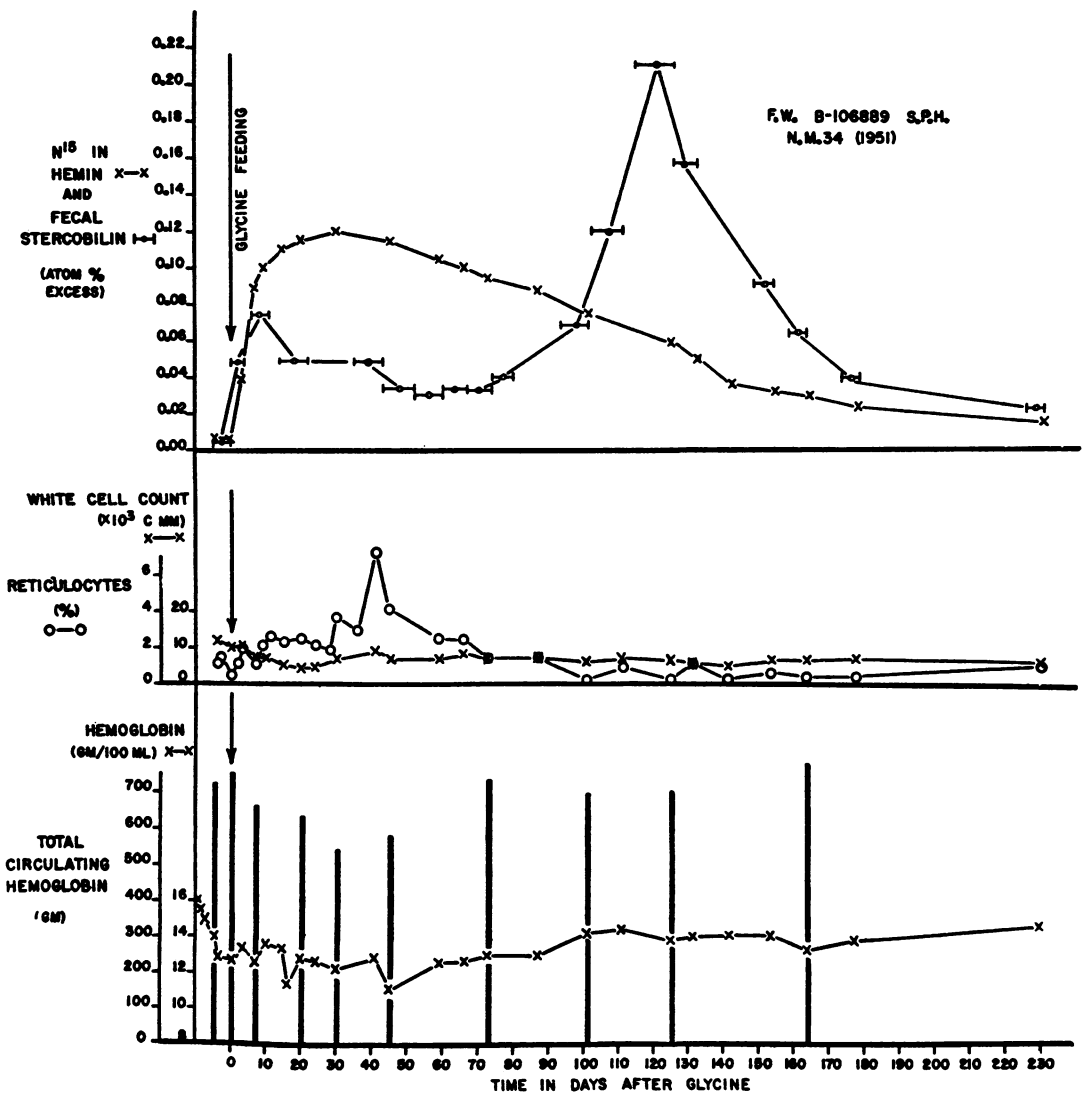

FIG. 5. INCORPORATION OF ISOTOPIC NITROGEN INTO HEME AND ITS APPEARANCE IN FECAL STERCOBILIN IN A BURNED MAN GIVEN N^{15}-GLYCINE 11 DAYS AFTER INJURY: CORRELATION WITH CHANGES IN HEMOGLOBIN, LEUCOCYTES AND RETICULOCYTES

lasted almost twelve hours, and although it was suspected he might have had some duodenal ulceration (Curling's ulcer), this was not proven. This difficulty did not cause any further discomfort. At no time during the treatment of the burn was he given transfusions, and he was skin grafted without mishap. During his hospitalization he was fed a liquid diet of the following composition: Protein 112 Gm. (1.5 Gm. per Kg.); carbohydrate 493 Gm.; fat 113 Gm. The caloric intake was 3435 calories per day. The use of similar diets in nutritional and metabolic studies has been described elsewhere (18).

Figure 5 shows, in the same manner as used with the animals, the changes observed in the uptake of heavy nitrogen into heme of hemoglobin in the burned man. Maximum uptake had occurred 30 days after the administration of the glycine, which was about 40 days after he had received the injury. Throughout the entire period of uptake the total circulating hemoglobin was decreasing, although the T.C.H. at the time of glycine feeding was not diminished.

In a normal man, studied twice in our laboratory, the maximum uptake occurred on the 15th and 20th days, respectively (29). During the feeding period the protein content of the diet had been reduced to less than 20 Gm. per day. These two men are not absolutely comparable, but, with the same amount of glycine, the normal man had incorporated at the 15th day almost three times as much heavy nitrogen in heme as had the burned man for the same interval of time. Maximum uptake into heme of the burned patient was 2.4 mg. N^{15} (0.16 per cent of administered N^{15}), while in the normal man it was 6.1 mg. N^{15} (0.42 per cent of administered N^{15}). The normal man's weight was 71.4 Kg. and that of the burned patient was 69.0 Kg.

TABLE V

Total circulating heme N^{15} in the burned man

Days after glycine	Hemin N^{15}	Total circulating hemoglobin	Total circulating heme N^{15}
	Atom % excess (corrected)*	*Gm.*	*mg.*
−4		731	
0		752	
7	0.084	664	1.93
20	0.111	632	2.43
30	0.115	537	2.14
45	0.111	585	2.25
73	0.091	743	2.34
101	0.072	685	1.71
125	0.056	705	1.37
164	0.026	772	0.70

* See footnote Table I.

The rate of uptake in the burned man does not seem to be greatly different from that which we have observed in the normal man with either normal or stimulated erythropoiesis. Blood letting of 20 to 30 ml. every 10 days was a constant stimulus to erythropoiesis. When a reticulocytosis of 7 per cent did appear between the 36th and 45th day, we did not observe any change in the N^{15} content of the hemin as was seen in the experimental animals. The best explanation for this is that any tag remaining in the body was now so diluted that it could not affect the tag already observed in the hemin.

After the 45th day there was a steady decline in the hemin tag which we attributed to slow dilution of the tagged cells with increasing quantities of untagged heme, or tag insufficient (*vide supra*) to affect it, during the period of regeneration of the total mass of circulating hemoglobin. The *total* circulating heme N^{15} calculated from the hemin N^{15} concentration and the T.C.H. showed little change from the 20th to the 73rd day as seen in Table V. This would appear to exclude excessive destruction of red cells up until the 73rd day as the cause for the decline in the hemin N^{15} curve.

Observe that the N^{15}-hemin disappearance curve seems to be almost a straight line from the 30th to the 125th day, when it changes its slope to the 142nd day, and then resumes a fairly constant slope to the 178th day. Because of these very slight changes in the tagged hemin disappearance curve it is difficult to select either by observation or by graphical methods the point of maximum breakdown of the tagged erythrocytes. The time of maximum decrease appears to be between the 125th and 142nd day, the mid-point of which would be the 133rd day. From analysis of the uptake curve the appearance of red cells with the maximum tag was about the 7th day, but tagging was taking place up to the 30th day. Therefore, from the hemin data the average life span of the tagged erythrocytes should be about 126 (133-7) days. It is interesting that these cells live a normal life span, and this observation in itself sup-

ports the proposition that the anemia is due to depressed erythropoiesis rather than to a shortened life span of the tagged cells. If the life span of the cells were unchanged, and the cells were produced while a non-hemolytic anemia was developing, then the anemia could only have been due to a diminished number of cells being formed. Similar reasoning was used by Berlin, Van Dyke, and Lotz in their study of the life span of the red blood cells in the hypophysectomized rat (30).

A more definite indication of the life span of the tagged cells comes from a consideration of the stercobilin N^{15} data which are plotted in the same figure. Consider that portion of the stercobilin curve from the 80th to the 175th day after glycine feeding. From a relatively constant level the N^{15} concentration in the fecal stercobilin began to rise at some time between the 80th and 93rd day and reached a maximum between the 114th and 125th day. Since the stools in this study were collected in eight-day periods for stercobilin isolation and analysis, the mid-points of collection periods were taken for the purpose of plotting the curve. Thus at the 120th day a maximum N^{15} concentration was found in the isolated stercobilin. At this time the average tag on the cells being destroyed was greatest. These cells were labelled during the period of glycine feeding. The stercobilin tag at this time was much greater than the circulating heme tag because the segment of the red cell population with the highest heme tag was being degraded to stercobilin. The average life span of the red cell from stercobilin data is about 120 days.

It has been demonstrated (31) from N^{15}-stercobilin studies in normal man that during the early phase of red cell labelling, the tag on the fecal stercobilin is greater than that which could be accounted for by breakdown of mature *circulating* erythrocytes. It was concluded that a portion, at least 11 per cent, of the stercobilin was not derived from circulating erythrocytes. In our patient, the average label on the stercobilin in the first four days was greater than that observed on the circulating hemin at the fourth day. It is also to be noted that the stercobilin label does not diminish to "normal abundance" levels between the 15th and the 80th days, similar to the results obtained by others (31) in normal man. Further investigation of this problem is now in progress in our laboratory.

There were continuous determinations of the urinary urobilinogen, coproporphyrin, and fecal urobilinogen, the results of which were not different from those presented in a previous study (1). At the time of glycine feeding and later, the fecal urobilinogen was not elevated.

Figure 6 gives the data relative to serum iron, copper, and free erythrocyte protoporphyrin, with which are correlated the changes seen in hemoglobin. There was present, within the first 10 days after injury, a hypercupremia and a hypoferremia. The significance of this distinctive pattern which is seen not only in iron deficiency anemia, anemia of infection (24, 32), rheumatoid arthritis, pregnancy, Hodgkin's disease, and leukemia is not known. To our knowledge, however, a serial observation through a *complete* phase, has not been reported previously. Contrasting the changes in the serum copper and serum iron with total circulating hemoglobin, the serum iron remained depressed for about 150 days, long after the T.C.H. had returned to normal. Serum copper on the other hand began to diminish toward normal levels, concomitantly with a rise in T.C.H. and a month before any possible trend in the serum iron was noted. Despite many recent studies, the significance of the role of serum copper remains unknown (33, 34).

The change in the free erythrocyte protoporphyrin is striking and has been observed in the anemia of infection (24). Shortly after the injury the EP began to rise and rose steadily to about the 54th day. The highest point was associated with a reticulocytosis of 7 per cent on the same day. The increase in EP without significant reticulocytosis from the 10th to the 50th day indicates reticulocytosis during this time was not directly related to the rise of the EP. We also have evidence that iron deficiency was not developing, since the mean corpuscular hemoglobin concentration shows no change in the 50-day period when the EP was rising rapidly. Iron deficiency causes the greatest increases in EP.

This very interesting observation suggests that the internal environment of red cells produced in this period of the burn was impaired, assuming that an increased quantity of free erythrocyte pro-

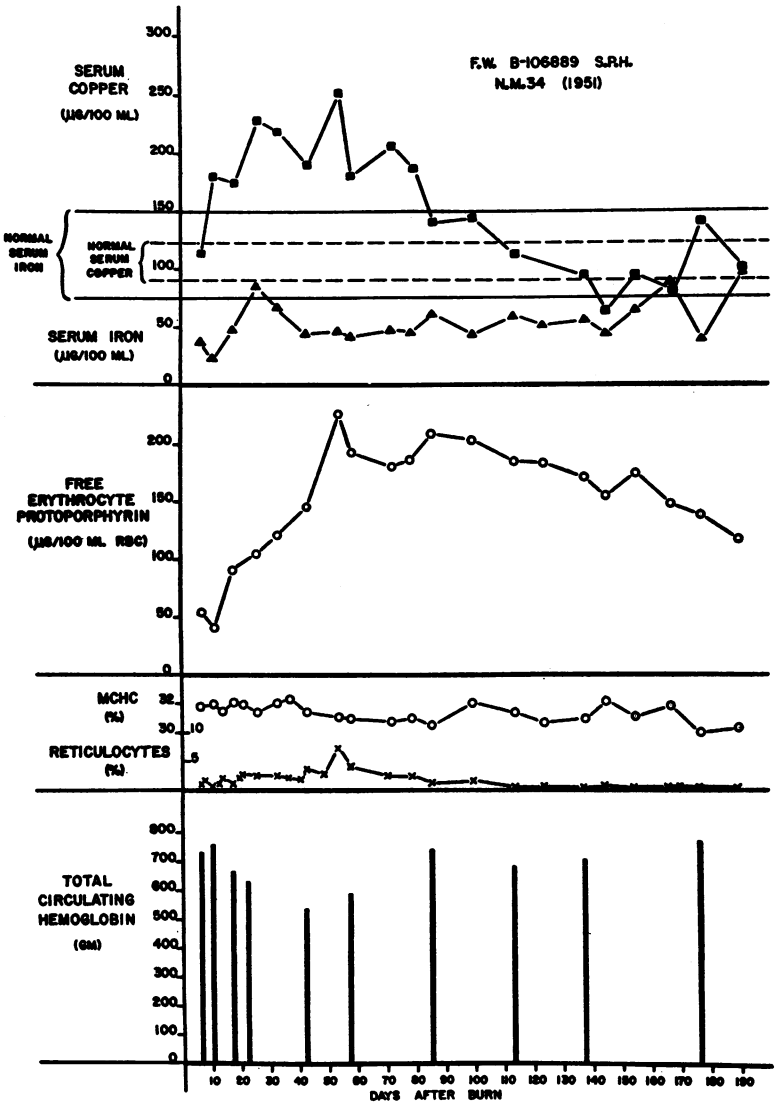

FIG. 6. CHANGES IN SERUM IRON AND COPPER, AND FREE ERYTHROCYTE PROTOPORPHYRIN, IN MAN AFTER THERMAL INJURY AND DURING RECOVERY

toporphyrin means a change in enzymatic reactions. From about the 50th to the 155th day, the EP remained relatively constant, and then began to diminish slowly. It was still high, even when the total circulating hemoglobin had returned to normal and when the serum copper and serum iron had returned to normal. The most plausible explanation for this is the production of abnormal red cells during the 50 to 60 day period, and so, these cells with a normal life span, as shown from the hemin data, would remain in the circulation with elevated EP for 120 plus 50, or up to 170 days.

SUMMARY

1. As measured by the incorporation of heavy nitrogen from orally-fed N^{15}-glycine into the heme of hemoglobin, a 20 per cent contact burn in dogs resulted in marked depression of the extent and rate of hemoglobin formation, ten days after the injury. At this time the total circulating hemoglobin was diminishing rapidly, but even this stimulus was not sufficient to cause a reticulocytosis.

2. By the hemin N^{15}-tagging method, the life span of the normal dog's erythrocytes was esti-

mated to be about 100 and 104 days, respectively, in two different animals.

3. In burned dogs no significant changes were observed in free erythrocyte protoporphyrin or serum iron and copper, but increases in fecal urobilinogen and urinary coproporphyrin excretions were similar to the changes observed in the burned man.

4. In a man, after a third degree burn of about 16 per cent, there was evidence of depression of hemoglobin formation when estimated by the extent of incorporation of heavy nitrogen from orally-fed N^{15}-glycine into heme. The life span of the cells formed during injury was estimated to be about 126 days. The isotopically-labelled fecal stercobilin excreted by this man demonstrated maximum destruction of tagged erythrocytes at about 120 days.

5. Free erythrocyte protoporphyrin, and serum iron and copper, followed a characteristic pattern in the burned man, the significance of which is unknown. Serial studies are presented for the complete phase after thermal injury and during recovery.

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