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GReprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 59, No. 1, February, 1922STUDIES IN THE REGENERATION OF BLOOD¹ZALIA JENCKS *Gayley**From the Sheffield Laboratory of Physiological Chemistry, Yale University,
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Studies in the regeneration of blood after simple anemia have been made on many species of animals and on man. Although various phases of the growth of blood have been extensively investigated in connection with destruction of the erythrocytes by chemical agents or specific diseases, much less information is available regarding recovery from the anemias produced by hemorrhage.

Many, if not most, of the earlier researches lack a convincing character because relatively few of the variables have been sufficiently taken into account. Heretofore the work on blood regeneration has been on animals whose dietary control was not complete. Inasmuch as the more recent investigations of nutrition, conducted on laboratory animals, have made it possible to control a variety of dietary factors, only one variable will need to be taken into consideration for the duration of a particular series of experiments.

The present studies on albino rats were undertaken because of the relatively large amount of information available concerning the dietary requirements of these animals. The rat is small enough to make possible the use of large numbers for experimental purposes and it has been employed as a laboratory animal for so many generations that laboratory conditions do not make its confinement unnatural. Our rats were from the colony of the Connecticut Agricultural Experiment Station. They were kept on a standard synthetic diet² while in experi-

¹ Material for this paper was taken from the dissertation submitted for the degree of Doctor of Philosophy, Yale University, 1921.

² Standard diet used:

	per cent
Cornstarch.....	50.5
Casein.....	18.0
Lard.....	18.0
Washed butter.....	9.0
Salt mixture.....	4.5

mental use and during all of the time when they were not under direct observation. This eliminated complications arising from having the animals eat such complex mixtures as they would, had they been fed table scraps.

Technique. The preliminary experiments were designed to discover the limit of successful bleedings in the rat, the period for regeneration of blood after hemorrhages, and to find the effect of the dietary factors on such regeneration.

The rats on which hemorrhages were produced were kept in iron-wire cages with paraffined tin cups for water and porcelain cups for food. The methods and technique employed have been described by Miss Ferry (1). The standard synthetic diet developed by the work of Osborne and Mendel (2), (3), (4) and others was used as a basis for all the dietaries employed. Adult and young animals of both sexes were used, varying in average weight from 100 grams for the young to 300 grams for the adults. The tables of Donaldson (5) were carefully followed in estimating the blood volume of each experimental animal used.

Experimental technique. The blood of every rat was carefully examined for the number of erythrocytes and percentage of hemoglobin before any hemorrhages were made. The counts were checked in every case during the first 2 months until the operator had attained sufficient skill to make such duplications unnecessary. The method of blood count was the familiar one with a Thoma-Zeiss hemocytometer and the use of either Hayem's or Toisson's solution for blood dilution. Hemoglobin was determined by the colorimetric procedure of Cohen and Smith (6). Rat blood was used as a standard and its color was checked by fresh material at frequent intervals.

The sample of blood for examination was taken from a drop extruded when the end of the tail was snipped. The rat was placed in an open glass bottle, the bottom of which had been removed and a tin cap with a hole for the tail placed over the opening; leather straps from the neck of the bottle held the rat securely in this armor (see fig. 1). This arrangement allowed free manipulation of the tail by the experimenter. The skin of the tail was softened with mineral oil. Previous to removing the end of the rat's tail it was placed in warm water or brushed over with toluene in order to suffuse the blood vessels. There are four blood vessels in the rat's tail, one dorsal, one ventral and two lateral. The blood was obtained from these by manipulating the tail after the fashion of milking and allowing the fluid thus "milked" out to drop into a

tared glass dish. At the beginning of this series of experiments, hemorrhages of from one-third to one-half of the estimated total blood volume were produced. After "milking out" 20 or 30 drops a clot usually formed; this was carefully wiped off with a previously weighed piece of cotton and added to the blood in the dish. Then more blood was worked out of the end of the tail. The blood and vessel were weighed rapidly to centigrams—two or three times, if necessary—until at least one-third of the whole blood volume contained in the rat had been removed. This method which was laborious and unsatisfactory resulted in occasional injury to the tail accompanied in some cases by

Fig. 1. This shows a rat held in the bottle so that the tail is exposed for "milking" out blood.

infection. Frequently a second hemorrhage was desired before the wound on the tail was completely healed. Because of this soreness and the possibility of infection it often became impossible to obtain a sample of healthy blood for cell count and hemoglobin determination.

Subsequently, therefore, the method used for obtaining blood from guinea pigs in serum work was adopted.³ In this procedure blood is secured directly from the jugular vein. The rat was anesthetized with ether, the fur closely clipped from its neck, a slit about $\frac{1}{2}$ inch long made in the skin, the jugular exposed, a v-shaped nick made in the vein with

³ Thanks are due my co-worker, Dr. W. G. Karr, for his kindly suggestion as to use of this method and for his assistance in the first operations.

scissors, and the animal held over a weighed glass vessel into which the blood was allowed to drop. If care was exercised it trickled slowly and in a well-directed stream and scarcely touched the fur. The blood was allowed to flow into the dish up to the mark previously placed to indicate one-third the estimated total volume of blood. By the time this had run out a clot had usually formed. The clot was carefully and gently wiped off from the wound with a previously weighed bit of cotton, and this added to the blood in the dish, and the whole was then weighed. If a clot failed to form the bleeding was stopped by pressing a small (weighed) piece of cotton against the cut in the vein. Ordinarily this proved effective. If less than one-third of the (estimated) rat's blood had run out by the time a clot had formed, it was easy to start the bleeding again by harshly wiping the nick in the vein with a weighed pledget of cotton; then more blood dripped and a second weighing was made. This second bleeding was, however, seldom necessary. The wound in the skin was then washed with dilute alcohol (25 per cent) and sewed up. The rats recovered readily, the wound healing in from 2 to 3 days in every case.

Blood counts and hemoglobin determinations were made daily until the original values returned. During the period of regeneration fragmentary red cells were often seen in the blood, but as regeneration proceeded these became fewer in number and finally disappeared altogether. The number of leucocytes increased the first day or two after the hemorrhage, but not in great abundance. As shown by Lamson (7) the number of erythrocytes as well as the percentage of hemoglobin varies among the individuals, but the drop after hemorrhage in each case is comparable.

Criteria of regeneration. The return of the number of red blood corpuscles and the percentage of hemoglobin to the values noted before any loss of blood had been sustained by the rat were the criteria used in judging regeneration. These values remained approximately constant for two successive days before the rat was considered to have regenerated his blood. For normal animals of either sex fed on standard diet (see footnote 2) with the addition of dried brewery yeast, the time was from 6 to 9 days (table 1). This has been corroborated by the subsequent studies of Geiling and Green (8) in this laboratory. At the end of the period required for regeneration, in many cases a second hemorrhage required about the same time for a return to normal blood conditions. A third hemorrhage was made on some of these rats, but regeneration in such animals always needed more time. If, however,

a week's time intervened between return to normal and the following bleeding, the regeneration rate was normal. Similar experiences have been recorded by Geiling and Green for double hemorrhages.

Blood regeneration in growing rats. To study the effect of loss of blood on the growth of young animals bleedings were made on healthy growing rats fed on the standard diet and brewery yeast as the adults were fed, the hemorrhage procedure being the same as for the grown rats. The time of total regeneration for these little rats was from 7 to 9 days (see table 2). After the hemorrhage they recovered quickly,

TABLE 1

Blood regeneration in adult rats after a single hemorrhage on the standard diet

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES*	HEMOGLOBIN	TIME FOR REGENERATION
grams	grams	grams	per cmm.		days
220	130	5.5	9,360,000	91	
			10,136,000	104	7
210	12.5	5.5	9,472,000	100	
			9,168,000	87	9
272	15.7	5.5	8,256,000	94	
			9,008,000	87	8
310	17.5	7.0	9,264,000	104	
			9,504,000	91	6
250	14.5	5.1	9,500,000	110	
			9,104,000	98	7
210	12.4	5.0	9,000,000	94	
			9,100,000	96	7
300	17.4	5.0	9,664,000	107	
			8,800,000	91	8

* The figures in the tables under the heading Red Blood Corpuscles give the number of erythrocytes before hemorrhage and the number at the end of recovery.

continued to grow and increase their body weight in a normal manner. Apparently the loss of one-third of their blood did not hinder growth in any observable respect. Some young animals whose growth had previously been delayed by lack of vitamin B in experiments by Cajori (9) were also used, but these recovered in the same way as the animals showing uninhibited growth.

Diet and dietary factors. In order to make these experiments as accurate as possible and to eliminate some of the variables occurring in other work on blood regeneration, the standard diet, with the addition of dried brewery yeast, was used. Diets of each component of this

composite food were used, that is, protein only (casein); carbohydrate only (cornstarch); fat only (washed butter); protein-free food (butter, lard, cornstarch and salt mixture)⁴ and one food accessory (vitamin B) in a relatively concentrated form. The sources of the food accessory

TABLE 2

Blood regeneration in growing rats after a single hemorrhage on standard diet

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
92	6.1	2.1	11,264,000	100	
			8,500,000	100	9
95	6.3	2.1	9,536,000	100	
			7,752,000	96	9
85	6.0	2.25	10,880,000	110	
			9,312,000	110	9
111	7.2	2.4	8,256,000	94	
			7,442,000	96	7
112	7.2	2.4	10,336,000	114	
			9,440,000	98	7
103	6.77	3.0	9,504,000	110	
			8,448,000	94	7
113	7.2	2.5	10,000,000	110	
			9,109,000	110	7

TABLE 3

Blood regeneration in adult rats after a single hemorrhage on a mixed diet

DIET	BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
Dog biscuit.....	322	18.34	8.2	10,192,000	94	
				8,400,000	98	7
	320	18.0	6.3	10,080,000	100	
				8,912,000	98	7
Table scraps.....	310	17.7	5.25	8,812,000	78	
				8,800,000	96	10

were dried brewery yeast, yeast concentrate⁵ (10), tomato juice and orange juice.

⁴ Salt mixture, Osborne and Mendel, Journ. Biol. Chem., 1919, xxxvii, 572.

⁵ Yeast concentrate, Osborne and Wakeman (10).

Mixed diets. In order to learn what difference, if any, existed between the effect of our standard (synthetic) diet and that of scrap food on the rate of blood regeneration, two rats were fed on dog biscuit for 2 days before bleeding and then during the entire period of the manufacture of new blood. The results are shown in table 3. In both cases the organism regenerated hemoglobin more rapidly than red cells. This may have been merely accidental, but the same circumstances occurred in the case of one rat fed on table scraps (meat, lettuce, bread, etc.). This opens the question as to what factor or factors are here present which we did not have in the standard basal diet with added yeast as a source of vitamin B.

TABLE 4

Blood regeneration in adult rats after a single hemorrhage on a limited food intake—5 grams per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
292	16.7	5.8	10,000,000	91	
			9,128,000	90	7
320	18.3	6.4	8,256,000	94	
			8,480,000	90	7
240	14.0	4.8	9,360,000	91	
			8,886,000	91	7
230	13.4	4.6	9,472,000	100	
			9,776,000	104	6
200	11.9	4.0	7,328,000	70	
			8,128,000	72	6

Underfeeding. A series of hemorrhages was made on animals with a limited supply of food, i.e., 5 grams per animal per day, including 0.2 grams yeast daily added to the standard basal diet as above. This limited food consumption did not prolong the duration of regeneration appreciably, the normal being reached from 6 to 7 days after the initial loss of blood (table 4). Loss of body weight occurred in each case, as might be expected since the caloric intake was insufficient, but the animals remained healthy and active throughout the period of limited feeding.

Starvation. When animals were not allowed any food throughout the period of blood regeneration, the rate of return to normal hemoglobin value and number of erythrocytes was slower than in the above cases, the period for one animal being as long as 13 days. A control rat with-

out hemorrhage was starved at the same time; its blood was found to vary no more in red cell count and hemoglobin percentage from day to day than that of a normal animal eating as much as it desired. Normal rats do not maintain wholly constant values for either hemoglobin percentage or number of red cells. This slower rate of regeneration during inanition is in agreement with the results of Hooper and Whipple (11) on fasting dogs after simple anemia (table 5).

Protein only. Since casein is the protein used in our standard synthetic diet and has been shown to be an adequate protein (2), this was fed as a sole source of nourishment to another group after hemorrhage until blood regeneration was accomplished. Only 2 grams per day per animal were used after it was found that the rats refused to eat more than this amount in that period. This limited protein diet was used to see whether in accord with the experience gained with the protein-

TABLE 5

Blood regeneration in adult rats after a single hemorrhage; during this period they received no food

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
340	19.2	7.5	8,860,000	114	13
			8,900,000	110	
330	18.7	6.6	8,562,000	74	10
			9,728,000	87	

rich diet of dogs (11) the rate of regeneration was more rapid than on other diets of one food factor only.

The time of blood regeneration in this set of experiments was from 9 to 12 days, more rapid than in starvation but not so rapid as on some other equally limited diets (table 6). It must be borne in mind however that no food accessories (vitamins A and B) were present and hence the animals ate less than they otherwise would. The energy intake was obviously inadequate so that underfeeding complicated the problem. In all these feeding periods where the diets were limited in amount or kind, or both, the rats were more coprophagous than normal, no doubt in an effort to satisfy their desire for a feeling of fullness.

Fat only. In feeding the fat portion of the basal diet, butter washed free of salt was used in an amount corresponding to the daily weight of protein fed. The rats did not eat the butter readily and never ap-

peared so anxious for their food as did the animals on the other diets. The time required for regeneration of blood by this set of fat-fed animals was 9 days, not longer than for the protein-fed rats (table 7).

Carbohydrate only. Two grams of cornstarch, made in the form of a paste with water to prevent scattering, were fed daily to each of three rats after they had been bled. These animals required 12 days for

TABLE 6

*Blood regeneration in adult rats after a single hemorrhage on a protein diet—
2 grams casein per day*

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERA- TION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
290	16.7	7.7	10,656,000	96	
			10,032,000	96	9
180	11.9	4.25	11,072,000	104	
			10,500,000	105	12
153	10.2	3.9	10,432,000	100	
			9,600,000	100	12
158	10.56	3.0	10,336,000	104	
			10,112,000	104	9

TABLE 7

*Blood regeneration in adult rats after a single hemorrhage; on a diet of butter fat only
—2 grams per day*

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERA- TION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
295	17.0	7.0	8,528,000	98	
			9,136,000	98	9
355	20.0	9.0	8,480,000	76	
			7,800,000	80	9
165	11.0	4.0	10,416,000	91	
			9,440,000	80	9

complete regeneration. This is somewhat longer than for those on the protein diet, in agreement with the work on dogs receiving a carbohydrate-rich diet (11) (table 8).

Protein-free food. This food was made up after the formula for the standard diet with the casein replaced by corn starch, making a food containing 68.5 per cent corn starch, 18 per cent lard, 9.0 per cent butter and 4.5 per cent salts. Three animals were given 5 grams of

this protein-free food daily, hemorrhages were made and the process of blood regeneration watched by the methods used before. These rats replenished their blood supply in from 9 to 12 days (table 9), slightly more time being required in this series than in the case of the diets of fat only, but in one case less than with carbohydrate only. Yeast was fed with this protein-free food to the extent of 0.1 gram per day but the exceedingly small amount of protein therein evidently had little

TABLE 8

*Blood regeneration in adult rats after a single hemorrhage; on a carbohydrate diet—
2 grams cornstarch per day*

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
150	9.5	3.2	10,288,000	90	
			9,568,000	91	12
395	22.0	8.5	11,600,000	116	
			10,368,000	114	12
240	14.0	6.0	9,600,000	91	
			9,696,000	94	12

TABLE 9

*Blood regeneration in adult rats after a single hemorrhage; on a protein-free diet
—5 grams per day*

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
195	11.6	4.47	8,976,000	87	
			8,496,000	90	9
255	14.8	6.0	10,896,000	110	
			10,392,000	107	12
190	11.45	5.3	10,000,000	110	
			10,400,000	110	12

or no effect on the speed of blood-cell formation. In all cases it was impossible to keep the animals eating this ration without the addition of the dietary essential, vitamin B. This led to work on diets without added yeast, with yeast as a sole source of nourishment, and with other vitamin-containing products, such as tomato juice and orange juice alone.

Standard diet without yeast. Four rats were supplied with an unrestricted quantity of standard diet and an abundance of fresh water, but

no yeast, i.e., no vitamin B, was given to them; they were bled according to the regular procedure and their recovery was apparently normal. They ate less and less as time went on, as has been the case for all animals with lack of vitamin B (12), and they lost in weight. The return to normal blood values came in from 12 to 13 days; in three of the four cases hemoglobin values were low. This could not be due to lack of iron, for some iron was eaten in the salt mixture, although more iron would have been consumed had yeast also been added to the diet (table 10).

Yeast only. Because of these results with protein-free food and with other diets without yeast, a series of hemorrhages was made on rats which were fed nothing but yeast during the period of recovery. Ac-

TABLE 10

Blood regeneration in adult rats after a single hemorrhage; on the standard diet, without yeast

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERA- TION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
295	170	6.48	11,648,000	107	
			11,292,000	96	13
315	180	6.0	10,112,000	91	
			10,288,000	94	12
355	200	6.9	11,584,000	114	
			12,100,000	104	12
270	156	5.4	10,768,000	98	(Sick)
			10,068,000	85	13

cordingly four animals were selected which had responded in a normal manner to regeneration after experimental anemia several weeks previous to this time and each was again subjected to a hemorrhage. These rats were then fed 0.5 gram dried brewery yeast daily. They ate the yeast with great avidity as soon as it was offered. This amounted to practical starvation, and although they lost body weight the animals all regenerated their blood in from 6 to 8 days (table 11). In each case the hemoglobin values were equal to or exceeded the original. This group of animals regenerated their blood in less time than the group without food.

As a check for these results with yeast alone a group was given 0.5 gram protein (casein) daily to see if the small quantity of yeast protein was the stimulant to forming new blood or whether it was in truth the

food accessory in the yeast. The time required for these three animals to regenerate their blood was 7 to 10 days. The hemoglobin values were again low in two cases as for the animals which were fed no yeast (table 12). The casein contained no iron but the rats were in iron-wire cages and continually gnawed at the wires.

TABLE 11

*Blood regeneration in adult rats after a single hemorrhage; on a diet of yeast only—
0.5 gram per day*

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERA- TION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>		<i>days</i>
292	16.76	6.5	11,184,000	112	
			10,416,000	112	8
320	18.0	6.2	9,134,000	90	
			9,648,000	98	7
290	16.7	7.0	10,784,000	96	
			10,336,000	98	6
355	20.0	7.0	10,144,000	102	
			10,656,000	104	7

TABLE 12

*Blood regeneration in adult rats after a single hemorrhage; on a protein diet—
0.5 gram casein per day*

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERA- TION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
147	9.34	3.2	8,704,000	102	
			9,036,000	98	7
150	9.5	3.0	12,000,000	108	
			9,924,000*	104	7
157	10.5	4.1	11,040,000	87	
			11,770,000	91	10

* R.B.C. constant 3 days

Water soluble vitamin (B). Two products other than yeast, rich in this dietary essential, were used as a sole nutrient for a group of rats which had experienced hemorrhages. Orange juice, containing no iron (by analysis and according to Sherman (13)) and tomato juice containing no iron (by analysis and according to Sherman) were fed to two sets of animals. The orange juice was prepared by straining a

mass of crushed pulp and juice through four layers of clean cheesecloth. This resulted in a perfectly clear fluid which was measured out for feeding by means of a pipette. The tomato juice was the juice from canned tomatoes, from which the pulp and seeds were separated by

TABLE 13

Blood regeneration in adult rats after a single hemorrhage; on a diet of orange juice only—vitamin B—5 cc. per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
250	13.5	4.9	12,384,000	114	
			11,548,000	114	6
118	7.7	2.8	12,000,000	110	
			11,880,000	112	6
170	11.2	3.2	9,776,000	102	
			10,608,000	112	6
275	16.	5.3	10,528,000	107	
			10,608,000	102	6

TABLE 14

Blood regeneration in adult rats after a single hemorrhage; on a diet of tomato juice only—5 cc. per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cmm.</i>		<i>days</i>
400	22.3	5.5	10,768,000	104	
			11,682,000	107	6
225	13.0	4.7	10,192,000	100	
			10,176,000	100	6
367	20.5	8.7	10,808,000	100	
			10,880,000	96	8
305	17.5	5.6	8,592,000	94	
			8,352,000	92	6

straining through four layers of cheesecloth. This was also measured with a pipette. The four rats on 10 cc. of orange juice daily, reached their normal blood values in 6 days (table 13), those on the tomato juice, 5 cc. daily, (they refused to eat as much as 10 cc. per day) recovered in from 6 to 8 days; again the hemoglobin values varied (table 14).

SUMMARY OF RESULTS

Hemorrhages of approximately one-third of the estimated total blood volume can be borne by albino rats without interfering with complete recovery. When one-half or more of the blood was removed by hemorrhage, death ensued. The criteria used in judging blood regeneration in the rat were the return of the number of erythrocytes and the percentage of hemoglobin to the values noted before the animal had sustained any loss of blood.

By varying the diet from elimination of one factor to elimination of all but one factor therein, the speed of regeneration of blood was changed, but with each diet the organism replenished its normal supply of blood to the original values for red cells and hemoglobin.

Protein permitted more rapid blood regeneration than either carbohydrate or fat when fed as a sole nutrient (2.0 grams daily).

The diets of vitamin-rich food, even in very small amounts, gave somewhat more speedy regeneration than any other diet containing one food factor only.

This study of blood regeneration has not covered the effect of iron on blood formation. Work on this phase of the problem is now being carried out.

I desire to express my sincere thanks to Prof. Lafayette B. Mendel who outlined this problem, for his suggestions and interest throughout the investigation.

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