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## Hemoglobin regeneration as influenced by diet and other factors\*

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Experiments usually have a past history or a genealogical sequence, and it may be appropriate at this time to review the genealogy of the *liver diet* experiments in anaemia due to loss of blood in dogs. With Dr. Sperry<sup>1</sup> in 1908 we took up a study of the liver injury produced by chloroform anaesthesia, giving particular attention to the regeneration of the liver cells to repair this injury. Icterus is invariably present in dogs with liver injury of this character and this condition was studied further. With Dr. King<sup>2</sup> we studied obstructive jaundice and found that the bile pigments were absorbed from the liver into the blood capillaries direct rather than by way of lymphatics. With Dr. Hooper in 1912 we began a systematic study of bile pigment production in the body as influenced by the Eck fistula<sup>3</sup>, and we were finally able to show<sup>4</sup> that hemoglobin could be rapidly changed into bile pigment within the circulation of the head and thorax, the liver being completely excluded; also that hemoglobin could be rapidly changed to bile pigment<sup>5</sup> within the pleural or peritoneal cavities.

After leaving Baltimore to work at the University of California (1914), Dr. Hooper and I<sup>6</sup> took up a careful study of bile pigment metabolism by means of bile fistulas in dogs and investigated the effect of diet upon bile pigment output. As these studies were continued<sup>7</sup> and extended to include bile fistulas combined with splenectomy and the Eck fistula<sup>8</sup>, it became apparent that we could not understand completely the story of bile pigment metabolism without more knowledge about the construction of blood hemoglobin in the body. Blood hemoglobin is a most important precursor of bile pigment and it was necessary to understand what factors influenced the building of new hemoglobin in the dog.

For this reason we produced simple anemia in dogs by means of blood withdrawal and in short experiments followed the curve of hemoglobin regeneration back to normal. These experiments with Dr. Hooper<sup>9</sup> were

\* This paper is designed to summarize the author's contributions but does not pretend to give a review in this field nor to describe the work of others.

begun in 1917 and it was found at once that diet had a significant influence on this type of blood regeneration. Because of our interest in liver function and injury<sup>10</sup> we soon began testing liver as one of the diet factors and could readily demonstrate that it had a powerful effect upon hemoglobin regeneration<sup>11</sup> (see Fig.1). These short anemia experiments were relatively crude and gave at best qualitative values for the various diet factors.

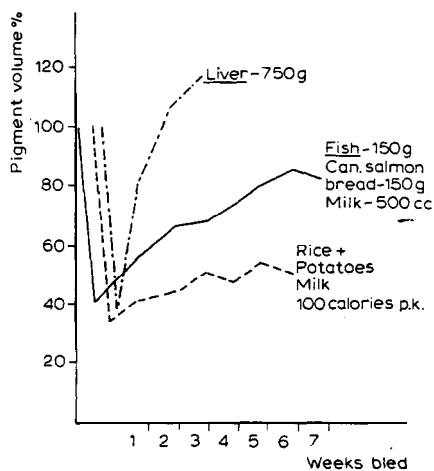


Fig. 1.

After the transfer of the anemia colony of dogs from San Francisco to Rochester, New York (1923), Dr. Frieda Robscheit-Robbins and the writer<sup>12</sup> began to use a different type of anemia. Dogs were bled by aspiration from the jugular vein and gradually reduced from a normal hemoglobin level of 140-150 per cent to about  $\frac{1}{3}$  normal, or 40-50 per cent, and this anaemia level was maintained a constant for indefinite periods by suitable removal of new-formed hemoglobin. The potency of the diet factor was then accurately measured in terms of the grams hemoglobin removed to preserve the constant anemia level. The stimulus presumably was maximal and uniform, and the reaction of a given dog to a diet factor was shown to be uniform when repeated time after time.

Much effort and time were spent in devising a basal ration adequate for health and maintenance during these long anemia periods lasting throughout the entire life of the dog (5-8 years). This salmon bread (Table 1)<sup>12</sup>, moreover, also permits of minimal new hemoglobin regeneration and therefore gives a low base-line hemoglobin output from which to measure the increased output due to liver, kidney, gizzard, or other favorable diet factor.

Table I. Composition of salmon bread.

<i>Ingredients</i> (g)		<i>Protein</i> (g)	<i>Fat</i> (g)	<i>Carbohydrate</i> (g)
Wheat flour	12,000	1,240	125	8,480
Potato starch	6,000			5,400
Bran	2,000	300	86	1,080
Sugar	3,000			3,000
Cod-liver oil	1,000	—	1,000	—
Canned tomatoes	2,000	24	4	80
Canned salmon	2,500	545	302	—
Yeast, compressed	455	55	2	96
Salt mixture*	150	—	—	—
Water	7,500	—	—	—
Total	—	2,164	1,519	18,136

*Protein*, 10.0 per cent. *Fat*, 6.5 per cent. *Carbohydrate*, 83.4 per cent. *Caloric value*, 4.8 per gram as fed.

\* McCollums' and Simmonds' salt mixture, with ferric citrate omitted.

From Table 2 it is obvious that liver<sup>13</sup> again stands out as the most potent diet factor. Kidney<sup>14</sup> is a close second. Gizzard, spleen, and pancreas also rate high as factors which favor abundant new hemoglobin production under these standard anemia conditions. Gradually various diet factors were standardized and this information was placed at the disposal of physicians who were concerned with the therapeutic treatment of human anemias. Iron<sup>15</sup> was found to be the most potent inorganic element.

Pernicious anemia, examined from the point of view of the pathologist<sup>16</sup>, was described in 1921 as a disease in which all pigment factors were present in the body in large excess but with a scarcity of stroma-building material or an abnormality of stroma-building cells. This fits quite closely with the modern conception of this interesting disease as developed from the important observations of Castle<sup>17</sup>. When the true factor is isolated I shall be surprised if it does not have to do with the stroma, but it may be related to the globin fabrication.

Hemoglobin utilization in anemia was studied in considerable detail. It was found that the anemic dog can conserve for new hemoglobin production about 100 per cent of injected hemoglobin<sup>18</sup>, whether given intravenously or intraperitoneally. Muscle hemoglobin was included in this study and there is a probability that some of the injected muscle hemoglobin is

Table 2. Hemoglobin potency of diet factors (average values).

Diet factor daily intake		Total net hemoglobin average output per 2 wks. (g)	Bread base-line hemoglobin average output per 1 wk. (g)	Hemoglobin output per 2 wks.		Number of expts.
				Maximal (g)	Minimal (g)	
Pig liver	300 g	93	6	124	69	77
Liver extract 55	300 eq.	56	4	72	37	22
Pig kidney	300 g	69	3	92	49	9
Beef heart	300 g	49	5	57	33	7
Apricots dried	100 g	42	4	92	13	31
Iron (Fe)	40 mg	53	6	95	25	43
Iron (Fe)	400 mg	94	7	127	67	6
Salt mixt.-Fe	6 mg	9	3	22	0	16
Salmon bread	400 mg	—	7	19	2	110

also used in this emergency to form new blood hemoglobin<sup>18</sup>. Certain digests of blood hemoglobin, when given intravenously, will be utilized to about 40 per cent to build new hemoglobin in the anemic dog<sup>18</sup>. Foreign hemoglobins (goose and sheep) are also readily utilized<sup>19</sup>, when given intravenously to the anemic animal, and we observe nearly 100 per cent conservation. Hemoglobin fed by mouth is poorly digested and we observe only about 10-15 per cent recovery as new-formed hemoglobin in anemia.

Liver fractions and extracts have been studied<sup>20</sup> and the active principles for this type of anemia separated from the active principle of pernicious anemia<sup>21</sup> as contained in the normal liver. The crude secondary anemia fraction<sup>20</sup> contains about 65-75 per cent of the potency of whole liver for new hemoglobin production in this type of experimental anemia and represents only 3 per cent of the whole liver weight.

Anemic dogs produce more new hemoglobin during a fast than during basal-diet periods; this phenomenon has received much study, with the hope that information relating to the internal metabolism of hemoglobin may be acquired. When a standard anemic dog is fed only on sugar plus iron, there will be a large output of new hemoglobin (100 g or more as a result of a two weeks' fast). Obviously this new hemoglobin must be derived from the body protein, and the mechanism of this reaction has been investigated by

Table 3. Hemoglobin construction and decrease in urinary nitrogen due to anemia and iron feeding.

Days on exper- iment	Fe intake (g)	Total N (mg/wk.)	Urea N	Urea N	Crea- nine N (mg/wk.)	Crea- tine N (mg/wk.)	Crea- tine	Uric acid N (mg/wk.)	Unde- ter- mined N (mg/wk.)
			+ NH <sub>3</sub> - N (mg/wk.)	+ NH <sub>3</sub> - N (%)			N + crea- tine N (%)		
Non-anemic dog 29-326.									
7	0	19,250	15,990	83.0	1,190	150	7.0	70	1,860
7	2.8	13,630	10,710	78.6	1,020	0	7.5	50	1,850
7	2.8	12,140	9,840	81.0	870	0	7.2	40	1,390
2	0	13,120	10,750	81.9	920	0	7.0	50	1,410
Anemic dog 29-326.									
7	0	25,550	21,320	83.5	1,150	460	6.3	150	2,480
7	2.8	13,830	10,450	75.5	970	50	7.3	120	2,250
5	2.8	11,420	8,180	71.6	770	360	9.9	70	2,040
2	0	11,550	8,230	71.2	770	360	9.8	70	2,120

Total hemoglobin production 112 g, equivalent to 19.0 g of nitrogen in anemic period.

Drs. Daft, Robscheit-Robbins, and Whipple<sup>22</sup>. Nitrogen partition of the urinary nitrogen shows that during such periods there is a conspicuous decrease in the urea-ammonia fraction, which points to a conservation of nitrogenous intermediates, which would otherwise appear as urinary N but under these circumstances are used to build new hemoglobin. The importance of this body reaction is obvious and it is being studied in considerable detail (Table 3).

Human liver material obtained at autopsy has been studied recently<sup>23</sup>, and its potency compared with standard animal liver material. If we rate pig liver as 100 per cent (our normal base-line), we may compare any given type of human liver with this control by means of our standardized anemic dogs. In this way it was found that the human liver from young healthy adults gives average values of 160 per cent. Elderly persons with arteriosclerosis and degenerative changes will show values for liver tissue of 117 per cent, as compared with the animal control of 100 per cent. Acute infections of course show swollen livers and this increase in size may account for the "dilution" of the active principle, but the average value for this liver tissue

is 117 per cent. Chronic infections give liver values which are practically normal (150 per cent). Cancer invasion of the liver reduces the values of the whole liver in proportion to the replacement by cancer tissue, which by itself appears to be inert. Liver cirrhosis is compatible with normal human values for the liver tissue, 164 per cent of the control animal liver, but when hepatic insufficiency supervenes these values drop markedly (48 per cent, or about  $\frac{1}{3}$  the human normal). Secondary anemia and leukemia show values somewhat below the human normal (125 per cent), indicating a moderate depletion of these reserve factors within the liver, presumably due to blood loss.

Pernicious and aplastic anemias show a definite heaping up of these potent factors within the liver tissue, which values run above 200 per cent. In aplastic anemia there is no formation of red cells; therefore the hemoglobin-building material piles up in reserve. In pernicious anemia there is a lack of something, so that the marrow cannot produce the needed red cells; therefore the hemoglobin-building material heaps up in the liver store-house (Tables 4 and 5).

Dogs with abnormal conditions are being included within the anemia colony and observations are accumulating to show in what measure splenec-

Table 4. Hemoglobin production factors in abnormal human liver - Pernicious anemia.

Number	Cause of death	Iron content human liver		Liver intake per day		Hemoglobin output per seven days' feeding		
		Fresh tissue (mg %)	Daily intake (mg)	Human (g)	Control (g)	From human (g)	From control (g)	Ratio to control (%)
A-371	No therapy	162.0	208	129	300	63	35	420
A-1800	No therapy	36.7	92	250	300	97	74	157
A-1045	Sl. therapy	47.3	130	290	300	112	56	208
X-2479	Nephritis	36.5	70	190	105	56	30	104
A-1472	Sl. therapy	17.5	27	158	300	52	50	200
A-425	Sl. therapy	34.8	52	150	300	45	34	265
A-1122	Embolism	24.6	33	130	300	25	30	192
A-1173	No therapy	—	—	160	300	37	46	148
	Average	51.3	87	182	—	—	—	218

Table 5. Hemoglobin production factors in human liver.

<i>Diagnosis</i>	<i>Cases no.</i>	<i>Average</i>	
		<i>Iron content (mg %)</i>	<i>Ratio human to control (%)</i>
Normal	9	12	162
Normal?	11	12	117
Acute infections	11	—	117
Chronic infections	16	12	149
C.P.C. liver	6	—	94
Amyloid—fat liver	10	—	111
Cancer liver	8	15	75
Cirrhosis	20	9	164
Hepatitis—insufficiency	10	10	48
Pernicious anemia	8	51	218
Aplastic anemia	4	70	201
Secondary anemia	10	7	135
Leukemia	14	13	129

tomy, the Eck fistula and the bile fistula may introduce factors having a bearing on the production of new hemoglobin under these standardized conditions. Acute and chronic infection, liver injury, and chronic nephritis are also being observed in the anemia colony. The list of abnormal states is a long one and includes disease conditions developing spontaneously as well as acute conditions of purely experimental nature. New hemoglobin regeneration can be influenced by many of these disease conditions, but it would be premature at this time to attempt evaluation of these effects. It is an interesting field, full of difficulties but also of promise for the future.

Amino acids deserve particular attention in this type of investigation and it should be possible to give certain amino acids intravenously and thereby influence hemoglobin production in anemia. We are proceeding with a systematic investigation of amino acids as diet factors in our standard anemic dogs. It would be premature to make any statement about amino acids at this time, but certain amino acids do exert a definite influence upon hemoglobin regeneration, when added in moderate amounts to the basal ration. Phenylalanine, tyrosin, and proline may be mentioned, but we have as yet no adequate data to establish any definite claim. The literature already con-

tains too many claims for the potency of one or another amino acid in anemia; the experimental data, however, are wholly inadequate.

It is obvious to any student of anemia that a beginning has been made, but our knowledge of pigment metabolism and hemoglobin regeneration is inadequate in every respect. This is a stimulating outlook for the numerous investigators in this field and we may confidently expect much progress in the near future.

1. G. H. Whipple and J. A. Sperry, *Bull. Johns Hopkins Hosp.*, 20 (1909) 278.
2. G. H. Whipple and J. H. King, *J. Exptl. Med.*, 13 (1911) 115.
3. G. H. Whipple and C. W. Hooper, *J. Exptl. Med.*, 17 (1913) 593.
4. G. H. Whipple and C. W. Hooper, *J. Exptl. Med.*, 17 (1913) 612.
5. C. W. Hooper and G. H. Whipple, *J. Exptl. Med.*, 23 (1916) 137.
6. C. W. Hooper and G. H. Whipple, *Am. J. Physiol.*, 40 (1916) 332.
7. G. H. Whipple and C. W. Hooper, *Am. J. Physiol.*, 42 (1917) 256.
8. C. W. Hooper and G. H. Whipple, *Am. J. Physiol.*, 43 (1917) 275.
9. C. W. Hooper and G. H. Whipple, *Am. J. Physiol.*, 45 (1918) 573.
10. N. C. Davis and G. H. Whipple, *Arch. Internal Med.*, 23 (1919) 612.
11. G. H. Whipple, C. W. Hooper, and F. S. Robscheit, *Am. J. Physiol.*, 53 (1920) 151 and 236.
12. G. H. Whipple and F. S. Robscheit-Robbins, *Am. J. Physiol.*, 72 (1925) 395.
13. F. S. Robscheit-Robbins and G. H. Whipple, *Am. J. Physiol.*, 72 (1925) 408.
14. F. S. Robscheit-Robbins and G. H. Whipple, *Am. J. Physiol.*, 79 (1927) 271.
15. G. H. Whipple and F. S. Robscheit-Robbins, *Am. J. Physiol.*, 72 (1925) 419.
16. G. H. Whipple, *Arch. Internal Med.*, 29 (1922) 711.
17. W. B. Castle, *Am. J. Med. Sci.*, 178 (1929) 748.
18. G. H. Whipple and F. S. Robscheit-Robbins, *Am. J. Physiol.*, 83 (1927) 60.
19. G. B. Taylor, E. J. Manwell, F. S. Robscheit-Robbins, and G. H. Whipple, *Am. J. Physiol.*, 92 (1930) 408.
20. G. H. Whipple, F. S. Robscheit-Robbins, and G. B. Walden, *Am. J. Med. Sci.*, 179 (1930) 628.
21. E. J. Cohn, G. R. Minot, G. A. Alles, and W. T. Salter, *J. Biol. Chem.*, 77 (1928) 325.
22. F. S. Daft, F. S. Robscheit-Robbins, and G. H. Whipple, *J. Biol. Chem.*, 103 (1933) 495.
23. G. H. Whipple and F. S. Robscheit-Robbins, *J. Exptl. Med.*, 57 (1933) 637.