STUDIES IN EXPERIMENTAL ANEMIA

I. The Effects on Rabbits of the Injection of the Hemolytic Toxin of the Welch Bacillus

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In this study the genesis of pernicious anemia has been investigated from several points of view. The first two papers are concerned with the hypothesis that pernicious anemia is perhaps caused by an intoxication arising in the intestinal canal. Experimental anemia was produced in the first instance by the hemolytic toxin of the Welch bacillus and in the second by the use of stool extracts from pernicious anemia patients and from normal individuals. The third paper is concerned with immunologic differences between pernicious anemia and anemia due to the Welch bacillus toxin. In a fourth paper experiments will be reported dealing with a change in atmospheric environment in which rabbits were exposed to three times the normal oxygen concentration in the inspired air.

HISTORY

During the past twenty-five years there has been presented a good deal of evidence pointing to the intestinal origin of pernicious anemia. Hunter (1) (1903) first definitely advanced the theory that pernicious anemia was due to a chronic infection of the gastro-intestinal tract, believing that this view was indicated by the specific glossitis, the gastric achylia and the intestinal symptoms characteristic of the disease. Kulbs (2), discovering that the intestinal contents of patients with chronic intestinal disorders contained hemolytic substances, believed the disease to be associated with hemolytic products of intestinal putrefaction. Herter (3) found that the stools of pernicious anemia patients contained greatly increased numbers of B. Welchii

and first suggested a relation between this organism and the genesis of pernicious anemia. The findings of Herter were confirmed by Simmonds (4) and recently by Moench, Kahn and Torrey (5).[1] In 1917 Bull and Pritchett (6) showed that B. Welchii elaborated a true hemolytic toxin. Intravenous injection of broth cultures into rabbits was attended by severe anemia and death. Intramuscular injection of like doses, however, caused death with equal certainty but without blood destruction. They were able in addition to produce an antitoxin which conferred immunity to the effects of the hemolytic toxin. Cornell (7) showed that rabbits infected with B. Welchii developed anemia of varying degree, characterized chiefly by anisocytosis. The color index showed irregular variations. In the presence of definite anemia it was less often below than at or above 1. The greatest depression of blood count occurred early in the disease and was always followed by a compensatory rise which the author interpreted as the response of a healthy hemopoietic system. By injecting the toxin of the Welch bacillus into monkeys Kahn and Torrey (8) produced anemia with a blood picture similar to that of pernicious anemia. The color index was increased above 1.0. Poikilocytosis and macrocytosis were of moderate degree, anisocytosis was marked. The anemia cleared up in about twenty days and was not reinduced by continued injection. Patterson and Kast (9) found anemia of the secondary type after injection of the Welch bacillus and the toxin into rabbits. It is of interest that Sapinoso, Berg and Jobling (10) found that repeated injections of distilled water caused a 40 to 50 per cent drop in the number of erythrocytes and a corresponding fall in hemoglobin. There was a slight to a moderate polychromatophilia and anisocytosis with occasional normoblasts. However, despite continued repeated injections, the blood count returned approximately to the normal figures present before injection.

[1] Since this paper was submitted Nye reports that the same increase in B. Welchii spores occurring in pernicious anemia is found in cases of gastric achylia without pernicious anemia. On the basis of his observations and the tendency of B. Welchii to form spores in alkaline media, be believes that the spore increase in pernicious anemia is secondary to the gastric achylia rather than indicative that pernicious anemia is caused by chronic intestinal infection with B. Welchii (Nye, R. N., J. Clin. Invest., 1927, iv, 71. Investigation Relative to B. Welchii Infection of the Intestinal Tract as the Etiological Factor in Pernicious Anemia).

Faber (11) has long been interested in the conception that pernicious anemia may arise from pathological disturbances in the intestinal tract. In 1895 he observed a case in which marked strictures of the small intestine were found at the postmortem examination. Meulengracht (12) reported a case of a woman 64 years of age who suffered from three strictures in a segment of the small intestine and in whom the signs and symtoms of pernicious anemia made their appearance. Similar observations have been made by others (Wallis, W. J. Mayo, Warfurnge, Ketz, P. F. Holts, Barker and Hunder, Schmidt, Tallquist). Faber refers the etiologic connection to resorption of hemotoxins of bacterial origin from the dilated section of intestine above the stricture. These results have apparently received experimental confirmation by the work of Seyderhelm (13). In two of ten dogs in whom circular strictures of the intestines were produced a progressive hyperchromic anemia of the pernicious anemia type was produced. In ten patients with pernicious anemia a preternatural anus was produced by an opening into the small intestine from which flowed a dark brown fecal fluid that could not be distinguished in appearance, odor or bacterial content from large intestinal stools. In those cases in which the small intestine fistula stool changed to its normal character, i.e., light color, absence of foul odor, no bacteria, there was a rapid improvement in anemia. With a special technique described below he made extracts of stools of pernicious anemia patients and normal individuals, both of which produced anemia of the pernicious type when injected into rabbits. Recently Dixon, Burns and Giffen (14) reported favorable results from ileostomy in patients with pernicious anemia. Autor (15) reported experiments in rabbits in which extracts of the entire bacterial flora of the stools of pernicious anemia patients and extracts of B. coli cultures from the same stools were repeatedly injected. The injections of the mixed bacterial extracts caused no changes in the blood picture; the B. coli extracts caused some anemia in a few of the animals which was not of the pernicious type.

Such reports as these appeared to justify a tentative hypothesis that a hemolytic substance is formed in and absorbed from the intestinal tract of patients with pernicious anemia. The nature of the substance is entirely unknown. The experiments reported in this

first paper deal with the administration of the hemolytic toxin of the Welch bacillus. The toxin has been given in various ways to rabbits over longer periods of time than have hitherto been employed.

It is evident from the work just cited that the hemolysin can constitute only one factor in the etiology of the disease. Only certain patients with stricture of the intestine develop pernicious anemia. In the various dog and rabbit experiments great variation in the frequency and severity of anemia occurred. Furthermore, in the Bothriocephalus type of pernicious anemia there is no question that the disease is due to the presence of the parasite, for expulsion of the worm is followed by recovery. Nevertheless, in Finland it is estimated that 10 to 20 per cent of the population are infected with Bothriocephalus, although according to Schaumann's (16) experience only a few per thousand get pernicious anemia. Clearly then, whatever the extrinsic hemolytic factor may be, an equally specific intrinsic individual constitutional factor must likewise be sought to explain the occurrence of the disease in some individuals and animals and not in others. Draper (17) in analyzing the constitutional types of various disease groups has come to the conclusion that pernicious anemia patients have not only distinctive physical traits but also a uniformly characteristic psychic pattern. From these observations he has developed the hypothesis that the pernicious anemia race represents an approach to the neutre or species type in which there has been an arrest of differentiation in psyche and soma. One could imagine that there might be a different penetrability of the walls of the intestine or a peculiar genetic fault in the bone marrow of such people which represent inner counterparts of those other observable differences.

We have attempted to devise experiments to test differences in constitutional reaction but the complexity and perhaps also biological fixity of the fully developed animal is such as to make this procedure difficult or impossible. A number of experiments were conducted in which normal and castrate rabbits were subjected to various hemolytic agents, such as the Welch bacillus toxin and hemolytic stool extracts. No differences could be observed between the castrate and the normal rabbits; these experiments were temporarily abandoned in favor of a more detailed investigation of the hemolytic agents on normal animals. In the fourth paper an attempt to produce an al-

tered constitutional reaction by changing the atmospheric environment will be reported. Up to the present time little work has been done from the point of view of increasing or decreasing an animal's susceptibility to disease by altering its individual constitution. Indeed, this may only be possible by breeding experiments or by insults to germ plasm or embryo. The obstacles to such investigations are obviously great but it is to be hoped that further interest will result in successful efforts in this direction.

The mere fact that such modification experiments are being suggested indicates our strong suspicion that the rabbits may represent a type of animal which is by nature incapable of developing pernicious anemia. This suspicion is of the same sort as that which is now well re-established, namely, that the constitution of the patient is as much an etiologic factor of his malady as the attacking force. Consequently, methods employed to change an unsusceptible constitution of that sort into a susceptible one, would be analagous, so far as its relationship to disease is concerned, to any device which might be used to shift the character or angle of impact of an external agent of disease.

METHODS

The isolation and cultivation of the Welch bacillus and the preparation of its toxin were carried out according to the standard methods described by previous workers. In some instances the organism was isolated by injecting a fecal suspension into the ear vein of a rabbit which was then killed, and the carcass allowed to remain at room temperature overnight. The heart's blood or the liver generally contained the organism in pure culture. In other instances a fecal suspension was placed in litmus milk which was then exposed to 60°C. for one hour and incubated at 37° for 24 hours. The organism once identified by its morphologic characteristics and its cultural reaction to growth in milk was grown in 1 per cent glucose muscle broth as made by Bull and Pritchett (6) or in veal broth (de Kruif (18)). The toxin was prepared by filtering the culture through a Berkefeldt filter. Its hemolytic titre was tested by mixing graded amounts of toxin with 1.0 cc. of a 5 per cent suspension of washed rabbit red blood cells.

Complete hemolysis of a 5 per cent suspension of red blood cells was usually produced by 0.1 cc. of toxin and slight hemolysis as low as 0.025 cc. The hemolytic activity varied greatly, however, in individual strains of Welch bacilli. As observed by Moench, Kahn and Torrey (5), strains from pernicious anemia patients were not necessarily more hemolytic than those isolated from normal individuals.

The deterioration of the hemolysin was progressive and rapid at room temperature, and to a less extent when kept in the ice box. It was adjusted to a pH of 7.2 and a salt concentration of 0.85 per cent. Recent work by Neill (19) has explained the inactivation of B. welchii hemolysin as an oxidation process. He has been able to reactivate the oxidized lysin by reducing agents such as sodium hydrosulphite. By this method it might be possible to keep the toxin at a standard hemolytic titre for long periods, or as he suggested by keeping small amounts of the toxin in sealed tubes. In our experiments the hemolytic power of the toxin was subject to considerable variation as deterioration occurred. However, we attempted to use the toxin that approximated the standard of titre given above.

The hemoglobin determinations were made in the beginning with the Sahli method and later with the Dare hemoglobinometer. In both instances the standards were checked from time to time with the oxygen capacity method of Van Slyke and Stadie (20).

For anaerobic culture the Novy jar with displacement of the air by hydrogen was generally used. At other times a heavy seal with melted vaseline was employed.

The pathological material was stained with hemotoxylin and eosin. The liver was additionally stained for hemosiderin, and the spinal cord with the scarlet red fat stain.

RESULTS

The blood picture of four control rabbits was studied for periods of six months to two years. The red blood count and hemoglobin were measured at intervals of one to four weeks during these periods. The charts of two of these rabbits are given. In the first (chart 1) 19 determinations were made in eight months. The red blood count was 5,500,000, the hemoglobin 80 per cent and the color index 0.73 for the greater part of this time. The individual variations which occurred between counts varied from 5 to 10 per cent of the mean figure. In the second (chart 2) 46 determinations were made in twenty-four months. As an approximate mean the red blood count may be taken as 5,300,000, the hemoglobin as 73 per cent and the color index as 0.69. In both cases there is tendency of the red blood count and hemoglobin to increase, and in the second there is a 10 per cent increase in the color index. In the second case larger variations in red blood count, hemoglobin, and color index have occurred than in any other normal in the series. Variations between successive determinations of 10 per cent are frequent, and 15 per cent not uncommon. Many factors are known which influence the red blood count and hemoglobin, such as

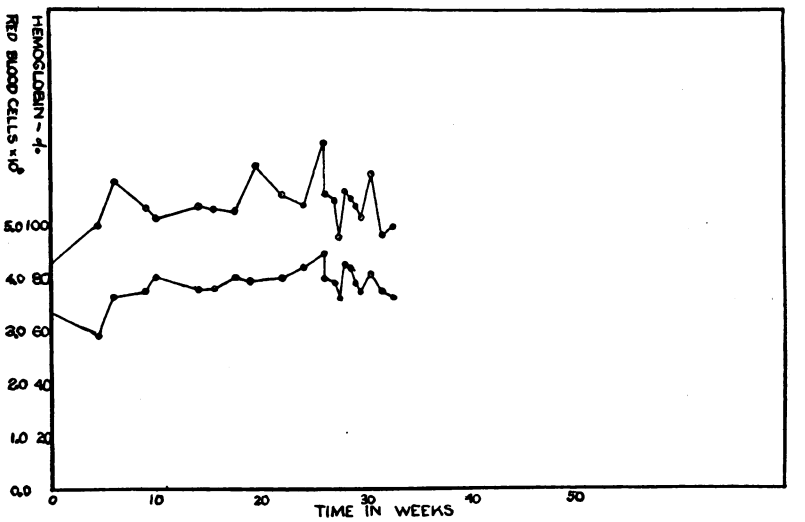

CHART 1. RED BLOOD CELLS AND HEMOGLOBIN OF A NORMAL RABBIT
Upper graph—red blood cells; lower graph—hemoglobin

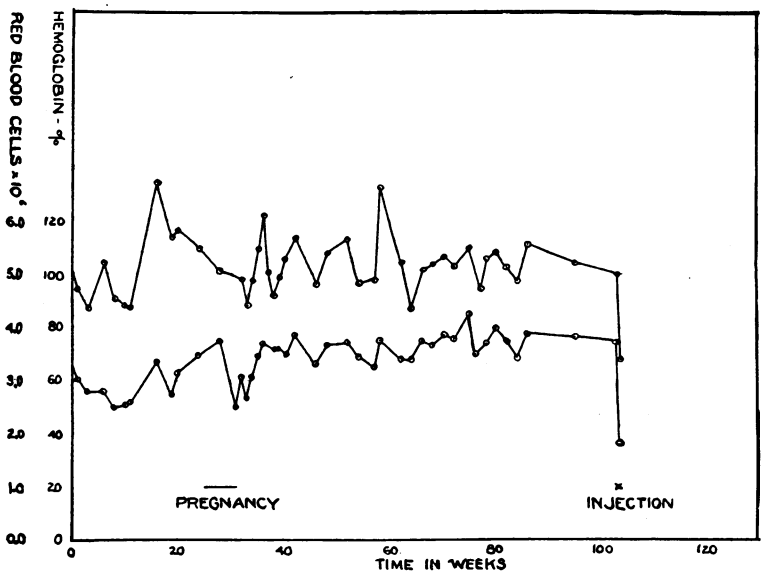

CHART 2. RED BLOOD CELLS AND HEMOGLOBIN OF A NORMAL RABBIT WHICH AFTER A CONTROL PERIOD OF TWO YEARS RECEIVED TWO INTRAVENOUS INJECTIONS OF WELCH BACILLUS TOXIN
Upper graph—red blood cells; lower graph—hemoglobin

excitement, time of day and relation to food. As far as possible the extraneous influence of these factors have been avoided in both the control and experimental animals. Such variations as do occur must be recognized and considered in connection with the experimental results. It is to be noted that the first rabbit had already attained adult life as indicated by a fairly constant weight, whereas the second rabbit underwent a progressive gain in weight as he became adult. This fact is probably to some extent an explanation of the greater variability in blood count of the latter animal. A fall in red blood

TABLE 1

Experiment 1. Anemia following single intravenous injection of Welch bacillus toxin

Date	Red blood cells	Hemoglobin	Color index	Remarks
	millions per cm.	*per cent*		
August 14, 1925.....	4.61	63	0.68*	Blood smear normal.
October 3, 1925.....	4.74	62	0.65†	
October 31, 1925.....	4.48	63	0.70	
November 11, 1925.....	4.40	63	0.71	
November 13, 1925.....	3.39	40	0.59	Marked anisocytosis, slight polychromatophilia
November 14, 1925.....	2.24	27	0.60	Marked aniso-; moderate poikilo-; and slight macrocytosis. Marked polychromatophilia
November 16, 1925.....	2.24	29	0.65	Died November 17, 1925

* One cubic centimeter Welch bacillus toxin injected intravenously on November 13, 1925. Blood count recorded 3 hours later.

† White blood cells rose from 7.400 to 37.800 on the following day.

cells and hemoglobin maximal during the thirty-second week of observation coincided with the termination of pregnancy. At the end of the control period of two years the rabbit received two intravenous injections of Welch bacillus toxin on successive days. The red blood cells dropped to 2,900,000, the hemoglobin to 36 per cent. The animal died on the day of the second injection.

Twenty-seven experiments were performed with Welch bacillus toxin. The toxin was injected intravenously, subcutaneously, intraperitoneally, and by a combination of these methods. Out of this

number eight will be chosen to exemplify the various types of response. Since the others were in the main confirmatory and because the data require so much space to publish, it seems best not to include them all in this report.

Experiment 1 (table 1). One cubic centimeter of B. Welchii toxin was injected into ear vein of rabbit. Three hours later the red blood count dropped from 4.40 million to 3.39, on the following day to 2.24, on the day after to 2.24. The hemoglobin dropped for the same periods as follows: 63, 40, 27, 29 per cent. The color index changed as follows: 0.71, 0.59, 0.60, 0.65. On the day after the last count the animal died. The white blood cells rose from 7,400 to 37,800 the day after the injection, and receded to 18,600 on the second day after the injection. Three hours after injection the blood smear showed slight polychromatophilia and marked anisocytosis; and on the following day, there was marked anisocytosis, slight poikilocytosis and polychromatophilia with numerous macrocytes. No nucleated red corpuscles were seen. The blood serum five minutes after injection showed the presence of hemolysis.

In this experiment a single intravenous injection of B. Welchii toxin initiated profound blood destruction and toxemia ending in the death of the animal. The blood picture was that of a severe secondary anemia due to active intravascular hemolysis.

Experiment 2 (table 2). Five cubic centimeters of Welch bacillus toxin were injected intravenously every two days for five doses, the toxin being of less hemolytic activity than that of the previous experiment. The maximum drop in blood count occurred nine days after injection as follows: Red blood cells from 5.41 millions to 2.78; hemoglobin from 70 to 38 per cent; color index changed from 0.65 to 0.69. (On two occasions during the anemia the color index was 0.80 and 0.82.) After the last injection the blood count rose again and continued to do so until it reached its normal figure forty-six days after the first injection. The blood smear at the height of anemia showed moderate anisocytosis and polychromatophilia. Later, the blood smear returned to normal.

This experiment indicates that non-fatal doses of B. Welchii toxin administered intravenously cause a secondary anemia which tends spontaneously to disappear.

Experiment 3 (table 3). One-half cubic centimeter of B. Welchii toxin was injected intravenously twice a week for a period of thirty-six days. The maximum anemia occurred six days after the first injection; the red blood count dropped from 5.63 millions to 3.46, the hemoglobin from 81 to 46 per cent, and the color index from 0.72 to 0.67. Notwithstanding repeated injections the blood count twelve days after the first injection came up: red blood cells to 4.64 millions, hemoglobin to 58 per cent, color index 0.63. Seventeen days after the last injec-

TABLE 2

Red blood cells and hemoglobin after five intravenous injections of Welch bacillus toxin

Date	Red blood cells	Hemoglobin	Color index	Weight	Remarks
	millions per cm.	*per cent*		*grams*	
January 14, 1926.....	6.08	75	0.62		Blood smear normal
January 28, 1926.....	5.70	69	0.61		
February 5, 1926.....	6.08	81	0.67	1,600	
February 18, 1926.....	5.50	79	0.72		
March 9, 1926.....	5.41	70	0.65	1,490*	
March 11, 1926.....	3.74	59	0.80	1,750	Slight anisocytosis and polychromatophilia
March 18, 1926.....	2.78	38	0.69		Moderate anisocytosis and polychromatophilia. Few macrocytes
March 24, 1926.....	3.90	64	0.82	†	
March 26, 1926.....	4.64	65	0.71		Slight anisocytosis
March 29, 1926.....	4.80	64	0.67		
April 6, 1926.....	5.22	67	0.64	1,850	
April 24, 1926.....	5.40	77	0.70	1,775	Smear normal
May 11, 1926.....	5.15	77	0.75	1,975	Smear normal

* Five cubic centimeter Welch bacillus toxin injected intravenously every 2 days.

† Injections stopped March 19, 1926.

tion the blood count dropped again: red blood cells 3.46 millions, hemoglobin 38 per cent, color index 0.55. During the periods of severe anemia there were marked anisocytosis and polychromatophilia and numerous macrocytes. The animal died the day after the last count.

A fairly severe anemia was produced from which the animal partially recovered notwithstanding repeated injections of toxin. However, twenty days after the last injection death occurred attended with a recurrence of severe anemia of the secondary type.

Experiment 4 (chart 3). In this experiment the rabbit was immunized by four subcutaneous injections of B. Welchii toxin, 3.0 cc. being given at the first dose, 5.0 cc. seven days later, and two 5 cc. doses at ten-day intervals. Two months later 5.0 cc. of toxin were administered subcutaneously three times a week for a subsequent period of fifty-two days without the development of anemia. Seven months after immunization 10 cc. of toxin were administered intraperitoneally three times a week for one month without the development of definite

TABLE 3

Anemia following intravenous injection of Welch bacillus toxin

Date	Red blood cells	Hemoglobin	Color index	Remarks
	millions per cm.	*per cent*		
November 25, 1925.....	5.63	81	72	Smear normal
December 3, 1925.....	3.46	46	67*	
December 4, 1925.....	3.68	50	74	Marked anisocytosis, polychromatophilia, and numerous macrocytes
December 9, 1925.....	4.64	58	63	
December 15, 1925.....	4.90	54	56	Slight anisocytosis
December 18, 1925.....	4.83	51	53	Slight anisocytosis
December 21, 1925.....	4.67	61	66	
December 30, 1925.....	4.64	58	63	
January 6, 1926.....	5.47	60	55	
January 8, 1926.....	4.90	60	62	
January 12, 1926.....	4.93	53	54	Slight anisocytosis
January 15, 1926.....	3.94	50	63	Marked anisocytosis, slight poikilocytosis and polychromatophilia
January 19, 1926.....	3.46	38	55	Same. Died January 20, 1926

anemia. Eight months after immunization 15 cc. of toxin were administered intraperitoneally three times a week for seven weeks. After a free interval of two weeks 10 cc. of toxin were administered intraperitoneally three times a week for six weeks; after a free interval of ten weeks 25 cc. toxin were administered intraperitoneally five times a week for five weeks. During the last two weeks of this course of injections the blood count gradually dropped, the red blood cells falling to 3.64 millions and the hemoglobin to 40 per cent with a color

index of 0.55. He then received two intravenous injections of 10 cc. each, and died after the last one without a final count being made.

In this experiment a rabbit was immunized by four small subcutaneous injections of Welch bacillus toxin. Thereafter for a period of over one year moderately large doses of Welch bacillus toxin failed to produce anemia. It appeared that each course of injections increased the animal's resistance to the toxin, for doses of toxin that readily produced anemia in normal animals were without effect in this

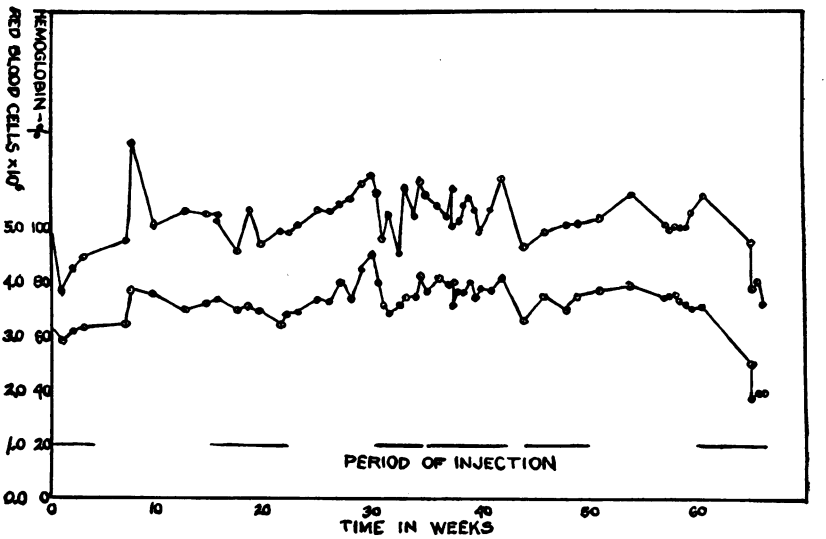

CHART 3. RED BLOOD CELLS AND HEMOGLOBIN FOLLOWING INJECTION OF WELCH BACILLUS TOXIN TO RABBIT PREVIOUSLY IMMUNIZED

Upper graph—red blood cells; lower graph—hemoglobin

instance. In the third paper it is shown that the blood serum of this rabbit had developed a strong anti-hemolysin. At the end, enormous intraperitoneal injections of toxin broke through the animal's resistance, and caused anemia and death.

Experiment 5 (chart 4). Five cubic centimeters of Welch bacillus toxin were injected subcutaneously into a castrated rabbit each day for four months. The maximum anemia developed thirty-seven days after the first injection: red blood cells dropping from 5.60 millions to 3.60, the hemoglobin from 69 to 35 per cent, the color index from 0.62 to

0.49. Notwithstanding repeated injection the blood count increased sixty-four days after the first injection to its normal value; red blood cells 5.30 million, hemoglobin 70 per cent, color index 0.66. A second period of injections of toxin in 10 cc. doses intraperitoneally three times a week from the thirtieth to the forty-first week of observation resulted in no anemia. A third and similar course of injections from the forty-ninth to the fifty-ninth week of observation likewise caused no anemia.

A fourth series of intraperitoneal injections beginning with 10 cc. doses and ending with 15 cc. doses three times a week from the sixty-

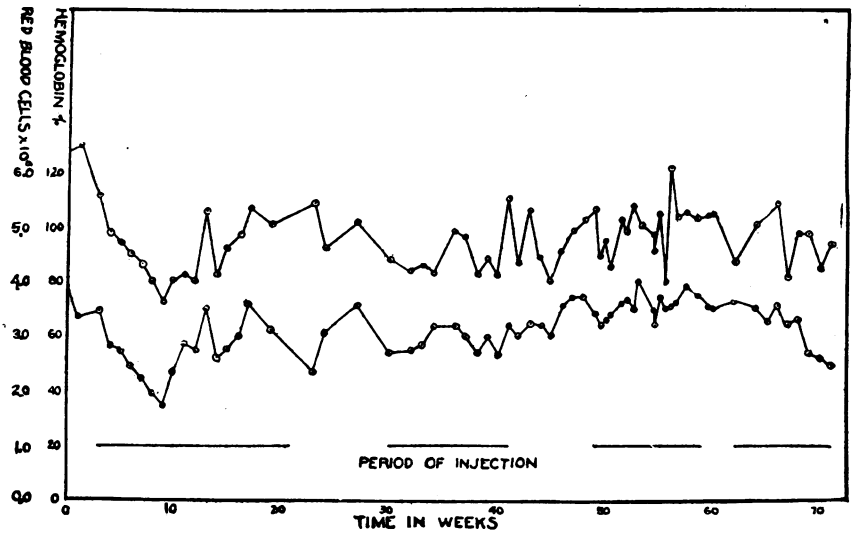

CHART 4. RED BLOOD CORPUSCLES AND HEMOGLOBIN FOLLOWING SUBCUTANEOUS AND INTRAPERITONEAL INJECTION OF WELCH BACILLUS TOXIN

Upper graph—red blood cells; lower graph—hemoglobin

second to the seventy-first week of observation resulted in a slight fall in blood count and death of the animal. Hemoglobin was 52 per cent, red blood cells 4.29 million, color index 0.68.

In this experiment a secondary anemia was gradually produced by the subcutaneous injection of Welch bacillus toxin. The blood count returned to normal while the injections were being continued. A second and third course of injections were without effect, and a fourth caused a slight drop in the blood count. As subsequent sero-

logical tests showed, the failure to produce a second severe anemia was due to the persistence of immune substances in the blood for a year following the first anemia.

Experiment 6 (chart 5). Five cubic centimeters of B. Welchii toxin and 2 cc. of B. Welchii antitoxin were mixed in a syringe and injected intravenously into a rabbit. Three hours later the red blood cells dropped from 4.51 millions to 3.22, the hemoglobin from 67 to 56 per cent, and color index changed from 0.74 to 0.87. Within the next nine days four similar doses were given, the count at that time being as

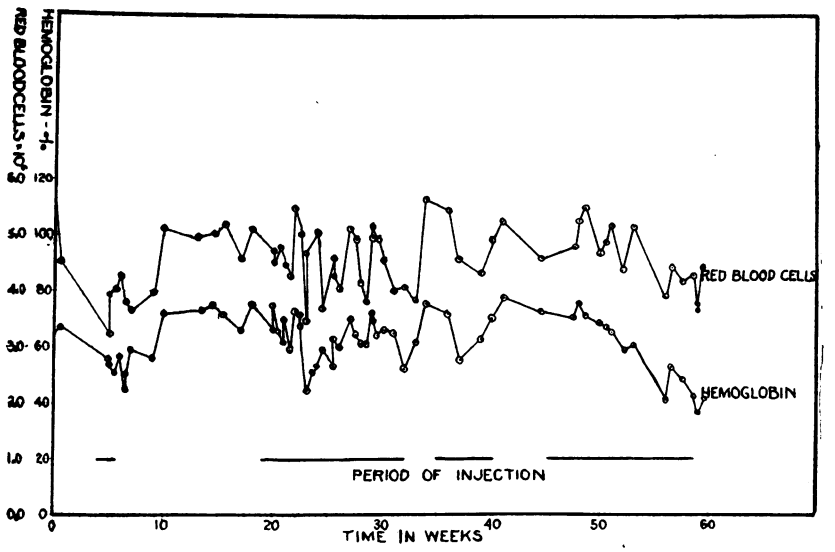

CHART 5. RED BLOOD CELLS AND HEMOGLOBIN FOLLOWING INTRAVENOUS AND INTRAPERITONEAL INJECTION OF WELCH BACILLUS TOXIN

follows: red blood cells, 3.74 millions, hemoglobin 45 per cent, color index 0.61. The blood smear showed marked anisocytosis and polychromatophilia. (Twenty-four days after the last injection the blood count had returned to normal.) Three months later 10 cc. toxin were administered intraperitoneally three times a week for six and a half weeks. At the end of three weeks the red blood cells dropped to 3.46 millions, the hemoglobin to 44 per cent, the color index to 0.64, but one month later the blood count was normal. After a free interval of three weeks a similar course of injections was given for five

weeks. Another drop in blood count occurred followed by a return to the normal. After a free interval of five weeks the same course of injection was given for seven weeks. Immediately after this 25 cc. of toxin were administered intraperitoneally three times a week for five weeks. The rabbit then received three intravenous injections of 10 cc. each on successive days, and died six days after the last injection. As a result of this final series of injections the blood count gradually dropped, reaching its lowest level three days after the terminal injection, red blood cells 3.66 millions, hemoglobin 36 per cent,

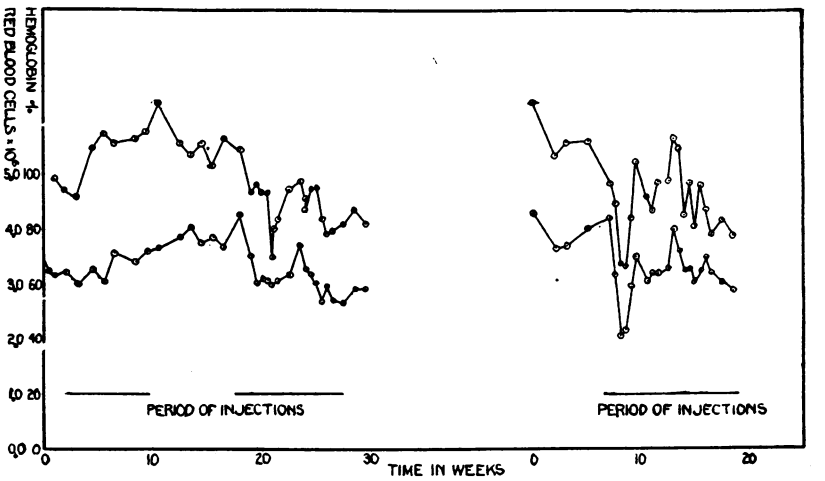

CHART 6. LEFT—RED BLOOD CORPUSCLES AND HEMOGLOBIN FOLLOWING SUBCUTANEOUS AND INTRAPERITONEAL INJECTION OF WELCH BACILLUS TOXIN. RIGHT—RABBIT NO. 166. SAME, FOLLOWING INTRAPERITONEAL INJECTION OF WELCH BACILLUS TOXIN

color index 0.49. The smear at the periods of anemia showed the same characteristics hitherto noted: marked anisocytosis, moderate polychromatophilia, slight poikilocytosis and macrocytosis, rare nucleated red corpuscles (always normoblasts when encountered). Chart 5 shows the changes noted above in relation to the periods of injection.

In this experiment carried over a period of one year four distinct periods of anemia were produced as a result of four courses of injection of Welch bacillus toxin, the first three followed by a remission

and the fourth by death of the animal. During the second long course there were frequent shifts from a low to a normal count. In the last course relatively large doses of toxin were needed to break down the animal's resistance. The anemia was of the secondary type.

Experiment 7 (chart 6). Five cubic centimeters B. Welchii toxin were given subcutaneously three times a week for three weeks, and then 10 cc. were administered in the same manner for three additional weeks without the development of anemia. Two months later 10 cc. of toxin were administered intraperitoneally three times a week for two and a half months. A slight anemia began sixteen days later, red blood cells falling from 5.14 millions to 3.49, hemoglobin from 85 to 59 per cent, color index changing from 0.79 to 0.85. One and a half months later the red blood cells had risen to 4.86 millions, the hemoglobin to 74 per cent, the color index was 0.76. Three weeks afterward the count again dropped, red blood cells 3.97 millions, hemoglobin 54 per cent, color index 0.68. Anemia of this mild character persisted, and the animal died one month later.

In this experiment the subcutaneous administration of Welch bacillus hemolysin failed to produce anemia whereas the intraperitoneal injection in similar doses produced a mild anemia which was maintained for the greater part of two months. This experiment together with others not reported indicates that of the three methods employed the subcutaneous administration of toxin is the least effective in causing anemia.

Experiment 8 (chart 6) (166). Ten cubic centimeters B. Welchii toxin were injected intraperitoneally three times a week for three weeks. After a free interval of one month a second course of injection was given in the same manner for four weeks. The maximum anemia developed seven days after the first injection; red blood cells dropping from 5.60 millions to 3.36 millions, the hemoglobin from 80 to 41 per cent, the color index from 0.71 to 0.61. The blood smear showed slight piokilocytosis and polychromatophilia, marked anisocytosis, and numerous macrocytes. Seventeen days after the first injection the red blood cells had returned to 5.22 millions, the hemoglobin to 70 per cent, the color index to 0.67. The blood count began to go down again during the second course of injections and on the last day of injection, the day before death was: red blood cells 3.90 millions, hemoglobin 58 per cent, color index 0.74.

In this experiment the intraperitoneal injection of toxin in two courses was responsible for the development of anemia each time, although less marked on the second occasion. The first anemia occurred more promptly and with smaller doses than the second. Presumably, the animal develops some immunity during the first period of injection which accounts for the difficulty of rendering him anemic the second or third time.

PATHOLOGY

The sections of the bone-marrow of anemic rabbits generally showed marked hyperplasia of the red cell elements. The liver frequently revealed small deposits of iron. The spinal cord did not show the degenerative changes associated with combined sclerosis and pernicious anemia. Other changes were irregularly observed such as leukocytic infiltration of the portal spaces of the liver, congestion of the liver or spleen, cloudy swelling and fatty degeneration of the parenchyma cells of the liver and the grey and white matter of the cord. The pathological histology of the organs studied was not characteristic of pernicious anemia but appeared to be consistent with blood destruction from an active hemotoxin.

DISCUSSION

Twenty-seven experiments with Welch bacillus toxin were carried out over a period of two years. Rabbits were injected intravenously, subcutaneously and intraperitoneally, and by a combination of these methods. Various degrees and types of anemia were produced, from a severe intravascular hemolysis fatal to the animal in three hours to a long continued remittent anemia of one year's duration.

The intravenous injection of strong Welch bacillus toxin results in severe anemia which may be fatal in several hours or several days. The serum gives evidence of hemolysis immediately after injection. The smear is that of a hemolytic secondary anemia, with anisocytosis constantly the predominating characteristic. Polychromatophilia is usually well marked, poikilocytosis and macrocytosis slight and nucleated red cells consistently rare. The color index is generally unaltered or it is lowered. Only infrequently and for short intervals has an increased color index been found. In the anemias that have

been of long duration and recurrent in character, the tendency has been for the color index to be decreased. The same picture is produced more gradually with the intraperitoneal injection of toxin, and with more difficulty necessitating larger doses, by the subcutaneous route.

When an anemia is produced and the animal survives, the blood count almost invariably returns to the normal. This usually takes place within three weeks, and occurs despite the continued injection of the same or larger doses of toxin. The animal evidently has now developed an increased resistence to Welch bacillus toxin. If enormous doses of toxin are administered intraperitoneally or intravenously when the animal is in this state, it is possible to break through the resistance and cause a recurrence of anemia. The subcutaneous administration of toxin does not produce anemia in an animal previously anemic in doses which are as large as one may conveniently employ in a rabbit. The second and third recurrence of anemia is usually shorter than the first, the blood count sometimes returning to the normal in four or five days. In some instances, the blood count may be made to fall progressively by the continuation of enormous doses resulting finally in death of the animal. At times, shortly before death of the rabbit the blood count rises markedly and may return almost to the normal value. Thus, death may be the result of a toxemia that is not associated with anemia. Usually, however, there is an anemia at the end.

If an animal is first immunized against Welch bacillus toxin by four small subcutaneous injections at approximately weekly intervals he is protected from good-sized doses of toxin for a year afterwards but may be made anemic by very large doses given at frequent intervals. It appears probable that the mechanism is a balance between the antihemolysin in the blood and the hemolysin administered. In the third paper of this series the presence of anti-hemolysin is demonstrated both in the anemic and in the non-anemic phases of poisoning from the Welch bacillus toxin. As a result of this swiftly occurring immunity an anemia with remissions may be produced which superficially resembles the cycles in pernicious anemia. One might speculate on the basis of these observations that the intermittent character of primary anemia might be dependent on variation in immunity on the part of the organism to an hemolytic toxin. However, in regard

to the acute and chronic anemia which it is possible to produce with Welch bacillus toxin our evidence points rather toward this being a typical secondary anemia due to intravascular hemolysis.

Anemia due to Welch bacillus toxin, no matter how administered or over how long a period, shows the characteristics of secondary anemia. The hemoglobin is generally lowered more than the red blood cells giving a low color index. The smear shows a conspicuous absence of nucleated red blood cells, of marked distortion in the shape of the cells and of a predominance of macrocytes. Anisocytosis and polychromatophilia are the outstanding findings. The pathological study of the bone-marrow and liver reveal such alterations as might be expected of any hemolytic agent. The spinal cord, stained with hemotoxylin-eosin and with fat stains, fails to show the degenerative changes frequently met with in pernicious anemia.

SUMMARY

Various types of acute and chronic anemia have been produced in the rabbit by the intravenous, subcutaneous and intraperitoneal injection of Welch bacillus hemotoxin.

The anemia tends spontaneously to disappear notwithstanding the continued injection of toxin. In some animals followed over long periods of time by means of very large doses repeated recurrences of anemia may be produced which superficially resemble the cycles in pernicious anemia. In other animals the blood count remains up notwithstanding a prolonged period of injection of toxin. Immunity to the Welch bacillus toxin is quickly produced in the rabbit, persists over a period of one year and is broken down only by large doses of toxin administered intravenously or intraperitoneally.

The character of the anemia suggests a secondary type due to intravascular hemolysis. Anisocytosis and polychromatophilia are the striking changes in the smear. Nucleated red blood cells and marked distortion of red blood cells are conspicuously rare. Macrocytosis is not predominant although common. The color index is unchanged or, more commonly, diminished. The section of the bone marrow and liver reveal the changes consistent with blood destruction. The spinal cord sections do not show the degenerative changes seen in combined sclerosis and pernicious anemia.

The failure to produce the typical changes of pernicious anemia by the injection of Welch bacillus toxin raises the question as to whether the rabbit species is constitutionally capable of developing the disease. We are only justified in saying that we have been unable to demonstrate any essential similarity between pernicious anemia and experimental Welch bacillus anemia in the rabbit.

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