STUDIES ON PERNICIOUS ANEMIA

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STUDIES ON PERNICIOUS ANEMIA

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Pernicious anemia was first described in 1855 by Addison[I], whose name it bears, as a very remarkable form of general anemia occuring without any discoverable cause in both sexes, generally, but not exclusively, beyond the middle period of life. Post mortem examinations failed to reveal any organic lesion which he considered adequate cause of such serious consequences.

About fifteen years later a German professor, Biermer[I], at Zurich, reported on fifteen cases of anemia which, in regard to clinical features and absence of morbid changes show no essential differences from Addison's anemia. He called it progressive pernicious anemia, and the disease was hailed by the Germans as a new disease and given the name, "Biermer's Anemia", by which it is still recognized in the German literature.

In a review of the existing opinions regarding these two descriptions in the Lancet of 1903[II], the following essential differences between the two descriptions are designated: Addison recognizes no cause, no wasting disease, previous loss of blood or no relation to social conditions, making it very clear that the etiology is obscure. Biermer, however, states that it is found amongst poor people in unhealthful surroundings, with a lack of nourishing food, often following chronic discharges, especially diarrhoea with or without gastric disturbances. He further claims that the type of spontaneous origin without clear

etiology is the exception. Biermer's anemia is regarded as a large class of anemias including secondaries, of which Addison's is one distinct type.

In 1885 at the University of Edinburgh, Wm. Hunter[3] became interested in these anemias and concentrated his efforts on their clarification. He spent several years of investigation of many anemias, and finally came to the conclusion that the amemia of Addison's description is caused by a poison absorbed from the gastro-intestinal tract, which he claims also accounts for the vomiting, diarrhea, lesions in the mouth and disturbances of liver functions--the liver taking over the burden of disposing of the products arising from the blood destruction. These investigations led him to further ascertain the nature of these gastro-intestinal changes which resulted in the explanation that this type of anemia is due to a chronic toxemia resulting from an infection of the gastro-intestinal tract, the chief source being a stomatitis which he found invariably associated with it, and which he regarded as the result of neglected cario-necrotic conditions of teeth. He found this infection to be of streptococcal origin, chiefly, and as a therapeutic measure hygienic care of the mouth was suggested.

[2]The following description is taken from the Lancet 1900, and summarizes his theory to that time; "Pernicious anemia is a chronic infective disease localized in the alimentary tract, caused by a definite infection of certain parts of the mucosa of the alimentary tract, chiefly the stomach, occasionally mouth and of the intestine. It is

characterized by (1) intermittent destruction of the blood and increasing anemia (as a result of the absorption of poisons into the blood) (2) periodic disturbance of the alimentary tract, chiefly of the stomach and intestine, as local effects of the infection on the alimentary canal; and (3) occasional toxemic attacks characterized by fever, sweatings, general nervous symptoms; not infrequently by effects, e. g. numbness, tingling, ataxia, absence of reflexes--denoting deeper nervous changes, such as peripheral neuritis, sclerosis of the cord".

Paul Ehrlich made some valuable contributions on cytological differentiation as early as 1800 when he reported on the blood findings in several anemia cases which came under his observation. By his staining methods, he was able to distinguish three irregular forms of erythrocytes, two nucleated, the normoblasts which are the same size as normal red blood cells, the megaloblasts, which are giant nucleated red blood cells, and a third type of red blood cell which he designates--poikiloblasten--corresponding to microcytes, but he claims these are seldom found. He further distinguishes the anemias according to the type of cells found. Nucleated red blood cells were found in all severe anemias regardless of origin, but the normoblastic type was found in secondary anemias and a great excess of the megaloblastic type in pernicious anemia, with normoblastic to a lesser degree. He also alludes to the fact that the megaloblastic type of red blood cell reproduction is constant in embryonic life. Ehrlich's contributions have been of inestimable value in

light of the fact that diagnosis of pernicious anemia is rarely even definitely established without a blood picture.

Addison's anemia is not confined to any race or location. It has been found in all the European countries, and in Asia and Africa as well as our own continent. It occurs in both sexes, although a higher percentage is being reported at present in men than in women. It usually makes its appearance in the fourth or fifth decade of life. The disease developes so slowly that no definite symptoms can be described at the onset. After its presence has become established, its external features are characterized by the lemmon yellow tint of the skin with the retention of well-preserved fat, accompanied by a slight loss in weight. This is in contrast to the emaciation of secondary anemias. An increasing languor developes which becomes extreme in a short time. Other symptoms which are shared with secondary anemias are muscular weakness, palpitation, headache, dyspnoea, vertigo and edema of the feet.

Among the specific symptoms, the most outstanding is the disturbance in the gastro-intestinal tract. Sore mouth and tongue are upheld by Wm. Hunter as a diagnostic feature, although Dr. Osler does not find this constant in his experience. Diarrhoea is reported at some stage in nearly all cases. The diminution of hydrochloric acid in the stomach usually progresses to achylia gastrica, and is universally considered one of the outstanding diagnostic features.

Degenerative changes in the spinal cord are associated with at least eighty per cent of pernicious anemia cases. Manifestations of cord involvement include numbness, tingling, and at times marked neuritic pains. Three types of nerve involvement are distinguished by Osler[5], and these are:

1. Cases in which the patient had no well-marked nervous involvement, but definite lesions were found in the spinal cord upon autopsy.

2. Cases in which spinal cord lesions were evident with the anemia. These present a postero-lateral sclerosis picture which may be of tabetic origin, lightning pains, girdle sensations areas of anaesthesia and loss of reflexes.

3. Nervous symptoms of a postero-lateral sclerosis type preceding the anemia. As the disease progresses, great mental depression developes, sometimes with delusions. However, the mental symptoms are not marked as a rule.

The characteristics of the blood picture are definitely established as essential in diagnosis of pernicious anemia. These consist of the following: Total quantity is dimished and abnormally fluid. Erythrocytes are greatly diminished in numbers, and may even be lowered to less than 1,000,000 in advanced stages. Hemaglobin is quantitatively reduced although it is relatively high producing a color index of over 1. Macrocytes measuring as large as 15μ and microcytes as small as 2μ are seen. A great variation

in shapes of red blood cells is also characteristic of pernicious anemia. Megaloblasts and normoblasts are present, and metachromasia and polychromatophilia are also constant features. The leukocytes are normal or diminished in number, polymorpho-nuclear neutrophiles are rarely reduced, although an occasxional increase in small mononuclear cells may be found. Myelocytes up to eight or ten per cent are frequently present. The blood platelet count is diminished, and may be as low as 100,000 with a subsequent delayed coagulation time.

Histological examination[6] of the marrow of larger and longer bones reveals a loss in the normal yellow color from absorption of fat, and in its place a bright red color. On microscopical examination of the red marrow, a great abundance of nucleated erythrocytes is found. These cells are of both megaloblastic and normoblastic type. Myelocytes and Charcot's crystals are also reported.

The prognosis for pernicious anemia is considered fatal by most investigators. However, Cabot[5] has reported several cases which he considered cured after six years. Cases which did not terminate fatally are thought to have been diagnosed in error by other authors.[6] Remissions may occur as frequently as six times, lasting from three months to four years, and during these periods the blood count becomes more nearly normal. Fewer nucleated cells are seen, the hemaglobin rises to normal proportions, and

the patient shows marked general improvement.

A blood picture resembling the characteristic picture of pernicious anemia has also been found in a group of other diseases with which anemia is associated. Among these are several of parasitic origin, especially those produced by dibothriocephalus latus, balantidium coli and the malarial parasites. Estrus equi, a parasite found in the stomach of the horse, has been regarded as the etiological factor in pernicious anemia of the horse. According to Osler,[5] extracts of these larvae have produced this picture, even when freed from the hemolytic lipoids such as are considered responsible for the anemia associated with bothriocephalus latus anemia. However, the etiology of pernicious anemia of the horse has not been definitely established. Rivers[22] reports that it belongs in the class of diseases caused by a filterable virus.

Pregnancy was considered a possible cause for pernicious anemia by many early investigators, and the anemia which developes after parturition shows a marked resemblance to this anemic picture. Some chemical poisonings have also produced typical pernicious anemia blood pictures, among them lead, arsenic, phosphorous, phenyl hydrazine, pyridene, and nitrobenzyl. Todd and Sanford[23] indicate that severe wasting diseases, such as nephritis, cirrhosis of the liver, and gastro-intestinal diseases are sometimes responsible for serious anemic conditions. Of the

infectious diseases, tuberculosis, syphilus and leprosy are cited by them. Severe anemic pictures may also be produced after chronic or acute hemorrhages, as in cases of hemorrhoids, gastric or uterine cancer or gastric ulcers.

The word amemia refers to a reduction in the amount of blood or the number of red blood cells, and several possibilities exist which might account for such a reduction in any type of anemia. Among these are (1) extensive loss of blood through hemorrhage (2) destruction of red blood cells (3) insufficient or defective hematogenesis, and (4) a combination of any of the above.

According to definitions of pernicious anemia by Osler,[5] Delafield and Prudden,[6] Hunter[2] and other authors, the presence of a hemolytic factor is assumed which aacounts for the extensive blood destruction in pernicious anemia. the microscopical examination of the bone marrow also reveals a faulty hematogenesis. The cause for these phenomena, however, is still obscure.

Several theories have been advanced in an attempt to account for these phenomena. In 1876 Cohnheim[7] published his interpretation of the cause as being a reversion of the bone marrow to the embryonic type as the primary lesion. He assumes that the red blood cells are less resistant to injurious agents, either physical or chemical, due to the weakened condition of the hematopoietic system.

Stockman, in 1895[8], advanced the following hemorrhagic theory: "Pernicious anemia is not a disease in itself, but is a high degree of anemia, usually following on numerous remote and predisposing causes, all of them well recognized as tending to produce the anemic state: that in certain individuals the anemia induced by these causes tends to bring on degenerative changes in the blood vessels, which lead in turn to the occurrence of numerous capillary hemorrhages all over the body: and that it is the persistence and long continued duration of these small internal bleedings, assisted often by larger external ones, which confers on certain cases of anemia the fatal, or "pernicious" or "progressive" character of the illness." He even goes so far as to say that many cases undoubtedly follow severe or prolonged hemorrhage, citing pregnancy, lactation, typhoid fever as preliminaries, and even including syphilis, mental shock and chlorosis and poor nutrition.

Wm. Hunter's[9] theory is based on the toxin production in the intestine which in turn causes excessive destruction of the blood in the portal circulation. To substantiate his theory he carried out some interesting experiments on the iron content of the kidney, liver and spleen of pernicious anemia, secondary anemias and normal individuals with the following results: In pernicious

anemia the liver and kidney combined contained 360 mg. of iron per 100 gms. of dried tissue, whereas in health he found only 84 mg. in liver and kidney combined and in secondary anemia only 83. mg. in liver and kidney combined. The iron content in the spleen in health contains six to eight times more iron than the liver and kidney, and in pernicious anemia this condition is reversed. This, he claims, indicates that the toxin is of a hemolytic nature and the hemoglobin is excreted by the liver; however, where blood destruction becomes excessive the liver cannot take care of the excretion of hemoglobin and it passes into the general circulation and is excreted by the convoluted tubules of the kidneys. This accounts for his making the liver and kidney determinations together. Hunter does not account for the alterations in bone marrow, however.

Bunting[1] reports a theory of Ehrlich and Lazarus which recognizes a hemolytic substance similar to that of bothriocephalus latus which is absorbed and acts on the circulating blood, producing hemolysis. Through its circulation it also acts on the bone marrow resulting in faulty hyperplasia. "They conclude that the anomaly of blood formation and increased blood destruction are coordinate results of the same cause."

This last view is upheld by Bunting,[1] who points out the following evidences as favoring this theory: (1)

the symmetrical lesions found in the spinal cord must be due to a toxin and not the anemia itself, since they are not found in the most severe types of secondary anemia. (2) The atrophy and degeneration of the gastric tubules might be due to a toxin and not to the anemia itself, and (3) the capillary hemorrhages resemble capillary hemorrhages resulting from such intoxications as snake venoms, where they are the result of an endotheliolytic principle.

It was necessary, now, in order to substantiate the toxin theory, to find an organism in the intestinal tract to which this toxin production could be ascribed, and as a result, a great deal of experimental data has been accumulated on comparative studies of the flora of gastro-intestinal tracts of individuals suffering from pernicious anemia, secondary anemias, and also of normal individuals. A brief summary of some of the experimental work is of interest at this time in leading to a discussion of the experimental work accomplished here.

Herter[10] in 1906 reported on a comparative bacterial examination of the flora of feces of normal children and normal adults and also on both in diseased conditions. He found a number of B. aerogenes capsulatus, identical to Welch and Nuttall's bacillus, present in the normal flora. In some diseased conditions he reported an increase in the number of welchii. This increase he said might be temporary or permanent, and might constitute a definite infection of the gastro-intestinal tract. In connection with this

he also found a reduction in the numbers of B, coli as shown by quantitative fermentation tests and stained smears. Herter also reported a moderate anemia with a fall in the hemoglobin in conditions where an increase in Welchii with accompanying decrease in B. Coli occurred. Furthermore, Welchii is prominently characterized by its ability to induce a characteristic type of putrefactive decomposition designated as a saccharo-butyric putrefaction, the products of which are CO_2H, Butyric acid, and NH_3. This type of fermentation was often found in pernicious anemia and secondary anemias by Herter. He also found in cases of advanced anemias that when an improvement developed in the blood picture, a reduction in the number of Welchii and an increase in number of B. Coli was concurrent with that improvement. This close association between the anemias and the Welchii infection of the gastro-intestinal tract lead to the assumption that this infection might be the cause of anemias. Herter was the first to designate a definite organism, and a great deal of work has been done on studying the relation of Welchii to pernicious anemia especially.

Several clinical cases of gas ganarene infection have been reported in which anemia was an associated feature. LeRoy[11] in 1903 reported on a case of Welchii infection in the neck which was accompanied by a rapid progressive anemia. The red blood cells dropped from 4,800,000 to 3,121,000 within a few days. Klotz and Holman[12] also reported on

cases of anemia in coal miners who were infected with B. Welchii. In these cases the red blood cells dropped as low as 2,170,000 with Hb of 73 per cent.

In their investigations on the fecal flora from thirty-three pernicious anemia cases, Moensch, Kahn and Torrey[13] reported finding the flora of the large intestine as being of a non-proteolytic fermentation type in which streptococci and welchii were particularly prominent. Contrary to Herter, however, these authors found a large number of viable B. coli, and at times acidophilus bacilli which they suggest might be a result of achylia gastrica. They also suggest that the conditions in the small intestine may be such as to permit the "elaboration of an exotoxin of neurotroxic propensities", and the intestinal mucosa at some levels may be permeable to such bacterial products.

The following quantitative increase was reported by Davidson[14] from his investigations of thirty pernicious anemia and thirty-three normal flora: Streptococci increased 100 to 1000-fold; Coli increased 1000 to 10,000-fold, and welchii increased 100,000 to 1,000,000-fold. He too claimed that this non-proteolytic type of fermentation flora, containing a high proportion of viable organisms, is typical of all pernicious anemias. He further claims that this flora is found in the small intestine, from where absorption takes place because the hydrogen concentration at this level is optimum for the development of vegetative

welchii bacilli which he found instead of spore forms. From operative cases he found the stomach and duodenum conbained a large number of organisms normally contained in the ileum and colon, probably as a result of achylia.

A repobt on the fecal matter from ten ileostomies was made by Seyderhelm,[15] who found a dark brown fluid with a high bacterial content and a foul odor, analogeoud to fecal matter from the large intestine, existing for the first day following the operation. A day or two following, the odor changed to a less obnoxious nature, and the character changed entirely to light brown, bringing at the same time a change in bacterial content. The large masses of coli were declining to normal, but indicanuria did not disappear. Neither did the progressive hemolysin.. He concluded that the contents of the small intestine in pernicious anemia very definitely resemble those of large intestine. In cases where death followed soon after the operation, the fecal content did not revert to the healthy normal state and appearance. He further states that the "giftquelle" which is normally found in the large intestine with no injurious effect is spread to the small intestine in pernicious anemia, where absorption by mucuous membrane is optimum and the danger of intoxication markedly increasdd. In one case where the anus praeternaturalis was opened and closed at various times during remissions and regressions,

it was finally closed at the peak of a remission and within five days the red blood count fell from 4,500,000 th about 1,000,000 and death ensued in a few days. On sectioning the tissues he found that during the Giftfreien Periode the parenchymal injury was völlig zurückgebildet.

These investigations point conclusively to welchii as a distinct possibility for the source of the toxin production. A comparison of the hemolytic properties of the three commonest organisms, streptococci, coli and welchii found in the intestinal tract resulted in the following report: streptococci were found to be very slightly hemolytic, coli no hemolysin production, but welchii were found to be markedly hemolytic. Cornell[16] injected sublethal doses of B. welchii into the spleens of rabbits and was able to produce a consistent anisocytosis varying in severity, similar to pernicious anemia, and a moderate poikilocytosis but not marked; whereas the hemolytic streptococci and B. coli failed to produce anemia.

Kahn and Torrey[17] attempted to injure the hematopoietic system of monkeys sufficiently to prevent remissions by intravenous inoculations of small amounts of welchii toxin. After three weeks, however, an immunity developed which could not be overcome by greatly increased doses and they concluded that it was impossible to damage the hematopoeitic system sufficiently in this way and that the condition was irreversible. They then injured the mucus membrane of the gastro-intestinal tract with sodium

fluoride and fed the monkeyd cultures of B. welchii via stomach tube. Severe anemia resulted, ultimately leading to death in a few weeks. The blood picture simulated that of pernicious anemia in the low red blood count, high color index, anisocytosis and at times poikilocytosis. Histologidal examinations revealed a desquamation of the cells of the stomach and the absence of any glands with intact secreting cells. The bones were found to be a dead white and brittle, the marrow softer and light red in color.

This work was followed by intramarrow innculations of the toxin by the same investigators.[18] A progressive anemia was produced which terminated fatally. A single inoculation into the tibial marrow brought on the anemia, loss in weight, and death, and also the possibility of the absorption of toxin as an etiological factor in that not only the inoculated bone was affected, but bones far removed also showed degeneration.

The immunological aspect of welchii toxin has not been so extensively studied. Herter refers[10] to several attempts to establish agglutinative tests which did not give consistent results. One investigator, Werner, was able to produce definitely positive results by employing a special technical procedure in the production of his immune serum in rabbits. He claims to have obtained agglutination up to 1:1000 dilution.

A toxin produced by Bull and Prittchett[19] was found by them to be thermolabile, being destroyed at 70 degrees centigrade for thirty minutes and diminished at 62 degrees. They also studied the antigenic properties by inducing an active immunity in pigeons through inoculations of carefully graded doses at weekly intervals for three weeks, after which two lethal doses of the toxic filtrate could be given with complete protection. They also produced an antiserum in rabbits which protected guinea pigs and pigeons against the toxin, whereas normal serum did not.

Cornell[16] found that when antitoxic horse serum, specific for welchii toxin of course, was allowed to stand with a welchii culture for two hours at room temperature and then inoculated into rabbits it failed to produce the destructive blood picture featuring anisocytosis so soon, the reaction being delayed from ten days to two weeks.

In 1927 Barach and Draper[20] reported on a series of interesting immunological experiments carried out by studying the hemotoxin of clinical and experimental anemia. The experimental anemia was produced by inoculating rabbits with B. welchii toxin producing anisocytosis. This type of anemia was characterized by remissions when the inoculations were carried over a long period of time. The blood picture returned to normal despite the continuation of the inoculations of the toxin, and the resistance could be broken down only by giving very large doses of toxin.

They assumed in this connection that the remissions in pernicious anemia were due to a varying immunity against the toxin, and also that the rabbit could not produce a typical human pernicious anemia. This led to an attempt to determine the immunological reactions associated with anemia due to welchii toxin in rabbits and the pernicious anemia in humans. This was done by studying the anti-hemotoxin. They found antihemotoxin present in the severest phase as well as in remissions in the sera of seven rabbits in whom chronic anemia had been produced. Rabbits rendered acutely anemic did not inhibit hemalysis, and neither did the sera of animals in whom anemia was developed for the second or third time. They claim this was due not to the disappearance of the antihemotoxin, but to the fact that the animal's resistance was broken down by the large doses of toxin. They found no inhibition of hemolysis in eight pernicious anemia cases in various stages ranging from severest to remission. From this work they concluded that the complete absence of an antihemotoxin in pernicious anemia patients studied offered no evidence that this disease has any relation to the experimental anemia in the rabbit, due to the toxin of the Welch bacillus.

EXPERIMENTAL DATA

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These studies on pernicious anemia were carried out with the following objects in view; (1) to determine whether pernicious anemia blood serum or normal blood serum possesses a protective element against welchii toxin, (2) to determine the resistance of red blood cells of pernicious anemia cases and normal individuals to hemolysis by a number of welchii toxins produced in various ways, (3) a study of the resistance of erythrocytes to physical trauma by using CO_2 and air under pressure in producing the trauma.

The normal individuals used in these experiments were secured largely from the bacteriology department. The pernicious anemia bloods were obtained from hospitals, and also from the private practice of several doctors. A total of nine cases were obtained, and one had to be discarded for inoculation experiments because it was not sterile. Following is a list of the pernicious anemia individuals with blood counts and hemoglobin estimations:

Table #1.

List of Pernicious Anemia Individuals.

Name	Date	R. B. C.	Hb.
1. P. A. Sanderson	4-11-29	4,200,000	85%
2. Norman Frisby	4-16-29	1,750,000	51%
3. Homer Bunch	4-19-29	1,330,000	25%
4. Cook County #1	4-26-29	3,720,000	80%
5. Cook County #2	4-23-29	2,610,000	60%
6. Mrs. John Frey	3-11-29	4,280,000	70%
7. Mrs. Deal	4-20-29	4,200,000	75%
8. Cook County #3	4-25-29	3,400,000	70%
9. Barber	-------	---------	---

Of these cases, numbers 2, 3, and 8 were in very bad condition, whereas the others varied considerably. Numbers 1, 6, and 7 show a high red blood count and were in a stage of remission at this time.

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To determine the presence, if any, of a protective element in the blood of pernicious anemia cases or normal individuals, sera were obtained under aseptic conditions. These were mixed separately with varying dilutions of toxin, ranging from just below the M. L. D. to just above it, and inoculated intraperitoneally into white mice. Each mouse was tagged, the inoculation mixture recorded, the weight of the mouse, and also the time of inoculation and death. The welchii toxin used was secured from the H. K. Mulford Company of Philadelphia, and labelled No.52. After careful titration it was found that .13cc of the toxin contained 1 M. L. D. To obtain greater accuracy a series was inoculated each time including .12cc, .13cc, and .14cc. The toxin was diluted 1.5 with physiological salt solution in each case, and to each dilution .5cc of serum was added. These mixtures were incubated 45 minutes at 37 degrees centigrade before inoculation. Protocol I indicates the method of making up these dilutions:

PROTOCOL I.

	Toxin	Saline	Total volume
a.	.12cc	.48cc	.6cc
b.	.13cc	.52cc	.65cc
c.	.14cc	.56cc	.7cc

Protocol II shows the method of making up the mixtures, and also indicates the series including the controls:

PROTOCOL II.

Toxin diluted as in Protocol I.	Serum, either P.A. or normal	Saline	Total Vol. inoculated
.6cc of (a) above	+ .5cc +	.1cc	1.2cc
.65cc of(b) above	+ .5cc +	.05cc	1.2cc
.7cc of (c) above	+ .5cc	-----	1.2cc
CONTROLS ---------	.5cc +	.7cc	1.2cc
.12cc	---- +	1.08cc	1.2cc
.13cc	---- +	1.07cc	1.2cc
.14cc	---- +	1.06cc	1.2cc

The above protocols were used in preparations for all white mouse inoculations. In order to eliminate the repitition of toxin controls, a series of two pernicious anemias and one or two normals was usually inoculated at the same time.

It was found very early that if any protection existed in the serum it was in too small a quantity to protect the mice against the lethal dose of toxin, and records were made to show any great variation in time of death after inoculation. The mice were usually inoculated

between five and six o'clock p. m., thus allowing 14 to 15 hours to elapse before eight a. m. the following day, when they were again examined.

A series of sera from eight pernicious anemia cases and from six normal individuals were used in this investigation. No marked protection could be demonstrated with either group. Those mice inoculated with .13cc and those inoculated with .14cc of toxin died regardless of whether or not they had been mixed with any blood serum or not. The mice inoculated with sublethal doses were not fatally affected, although they showed severe symptoms of toxic reactions. Some variation in the time which elapsed between inoculation and death was found in some instances, and is recorded in the following chart:

PERNICIOUS ANEMIA SERA

.12cc Toxin

P. A.	Time of inoculation	Time of death	Hours lived after inoculation
#1	5:10 p.m.	Did not die	Alive after 8 days
#2	5:00 p.m.	"	"
#3	5:20 p.m.	"	"
#4	7:00 p.m.	"	"
#5	7:50 p.m.	"	"
#6	4:50 p.m.	"	"
#7	4:30 p.m.	"	"
#8	5:00 p.m.	"	"

PERNICIOUS ANEMIA SERA

.13cc Toxin

P. A.	Time of Inoculation	Time of Death	Hours lived after inoculation
#1	5:15 p.m.	Before 8 a.m.	Less than 16 hrs.
#2	4-23-29 5:05 p.m.	4-25-29 10:00 a.m.	42 hrs.
#3	5:20 p.m.	Before 8 a.m.	Less than 16 hrs.
#4	7:00 p.m.	Before10 a.m.	Less than 15 hrs.
#5	5-9-29 7:55 p.m.	5-10-29 8:00 p.m.	24 hrs.
#6	4:55 p.m.	Before 8 a.m.	Less than 15 hrs.
#7	4:35 p.m.	Before 8 a.m.	Less than 15½ hrs.
#8	5:05 p.m.	Before 8 a.m.	Less than 15 hrs.

PERNICIOUS ANEMIA SERA

.14cc Toxin

P. A.	Time of inoculation	Time of Death	Hours lived after inoculation
#1	5:20 p.m.	Before 8 a.m.	Less than 16 hrs.
#2	4-23-29 5:10 p.m.	4-25-29 8:00 a.m.	40 hrs.
#3	5:30 p.m.	Before 8 a.m.	Less than 16 hrs.
#4	7:40 p.m.	Before 10a.m.	Less than 15 hrs.
#5	5-9-29 8:00 p.m.	5-10-29 8:00 p.m.	24 hrs.
#6	5:00 p.m.	8:00 a.m.	Less than 15 hrs.
#7	4:40 p.m.	Before 8 a.m.	Less than 15½ hrs.
#8	5:10 p.m.	Before 8 a.m.	Less than 15 hrs.

NORMAL SERA

Normal	Inoculation	Death	Hours lived
	-------With .12cc Toxin-------		
#1	4:50 p.m.	Did not die	Alive after 8 days
#2	5:25 p.m.	"	"
#3	8:00 p.m.	"	"
#4	5:00 p.m.	"	"
#5	6:00 p.m.	"	"
#6	5:10 p.m.	"	"
	-------With .13cc toxin-------		
#1	4:55 p.m.	Before 8 a.m.	Less than 16 hrs.
#2	5:30 p.m.	11:30 a.m.	18 hrs.
#3	8:05 p.m.	10:00 a.m.	14 hrs.
#4	5:05 p.m.	Before 9 a.m.	Less than 16 hrs.
#5	6:05 p.m.	Before 10a.m.	Less than 16 hrs.
#6	5:10 p.m.	9 a.m.	16 hrs.
		.14cc Toxin	
#1	5:00 p.m.	Before 8 a.m.	Less than 16 hrs.
#2	5:35 p.m.	noon	18hrs.
#3	8:10 p.m.	Before 8 a.m.	Less than 12 hrs.
#4	5:10 p.m.	Before 9 a.m.	Less than 16 hrs.
#5	6:10 p.m.	Before 10a.m.	Less than 16 hrs.
#6	5:20 p.m.	9:00 a.m.	16 hrs.

Normal serum #2 showed some delay in death over the other sera used.

Normal serum #2 showed some delay in death over the other sera used. Exact time in most cases could not be secured because the mice died during the night, but most of them died in less than sixteen hours. Normal #2 also gave interesting skin reactions in a guinea pig which will be discussed later.

A marked delay was found in two sets of mice inoculated with toxin mixed with pernicious anemia sera. The average lived less than sixteen hours. However, two showed a delay of eight hours and 24 hours. In both cases all mice inoculated with lethal doses died in about the same time, whereas the controls recovered. Number 5 showed a delay of eight hours, and had a red blood count of 2,610,000 with a hemoglobin estimation of 60%. The case which delayed death 24 hours had a red blood count of 1,750,000, with a hemoglobin estimation of 51%. Both of these cases showed more severe blood involvement than the other pernicious anemia cases, although one case, No. 3, with a red blood count of 1,330,000 and hemoglobin estimation of 25% did not show the same delay.

Since the white mice showed very slight, if any protection against the toxin, it was further decided to try some intracutaneous inoculations on the ventral surfaces of guinea pigs. Large white or tan guinea pigs were used for these skin tests. The ventral surfaces were carefully clipped and shaved at least six to eight hours before inoculations were made. The smallest amount of toxin

necessary to give definite reactions, consisting of reddening within 24 to 48 hours with subsequent ulceration and sloughing, was determined and used in all the mixtures with sera. This amount was found to be .05cc. The method for setting up the serum-toxin mixtures and the controls are shown in the following protocol:

PROTOCOL IV.

	Toxin	Serum	Saline	Total volume
(1)	.05cc	.05cc	0	.1cc
(2)	.05cc	.025cc	.025cc	.1cc
(3)	.05cc	.012cc	.038cc	.1cc
(4)	.05cc	0	.05cc	.1cc
(5)	0	.05cc	.05cc	.1cc
(6)	0	0	.1cc	.1cc

By having a large area and carefully arranging the non-toxic controls between the toxic mixtures it was possible to inoculate one guinea pig with six different mixtures. The case of the normal serum No. 2 showed interesting skin reactions which are shown in Figures I and II on the following page, actual photographs taken at the time of experimentation.

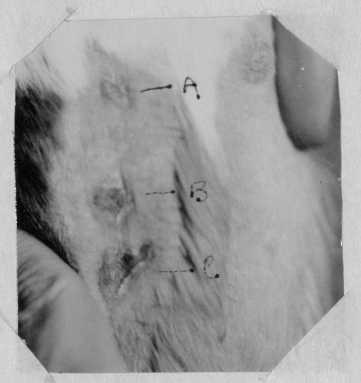

Figure I.

Figure II.

In figure I, A received .05cc toxin mixed with .05cc of serum No. 2; B received .05cc toxin plus .025cc serum No. 2; and C received .05cc toxin plus .012cc serum No. 2, corresponding to (1), (2) and (3) in Protocol IV.

Figure II shows the controls. D corresponds to the toxin control (4) in protocol IV; E corresponds to the serum control, and F indicates the site of the salt solution inoculation.

According to the results in the guinea pig skin reactions, where it is quite evident that .05cc serum showed some relative protection as compared to the toxin control D and also to the inoculations with smaller amounts of serum in B and C, and also the delayed death in the white mouse inoculations, a slight protection might be indicated in this normal serum. However, the other five normals showed no variations in reactions in white mice, and three others tested in guinea pigs did not show any protection at all.

One pernicious anemia case showed some protection as compared to another case with a correspondingly low red blood count. This occurred in P. A. 2 and 3. Instead of making up a group varying in amounts of serum used, it was decided to use the amount which had shown the greatest protection in previous cases, so the following protocol was used:

PROTOCOL V.

	Toxin	P. A. Serum	Saline	Total Vol.
(1)	.05cc	Frisby .05cc	0	.1cc
(2)	.05cc	Bunch .05cc	0	.1cc
(3)	. 0	Frisby .05cc	.05cc	.1cc
(4)	0	Bunch .05cc	.05cc	.1cc
(5)	.05cc	0	.05cc	.1cc
(6)	0	0	.1cc	.1cc

The following picturex indicates the reactions obtained from the inoculations made according to the above protocol:

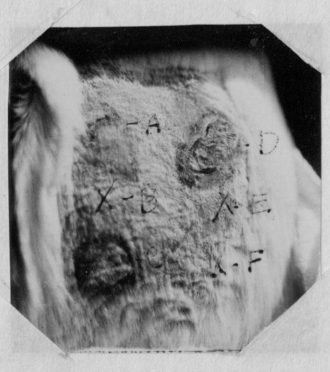

Figure III.

In Figure III, A corresponds to (1) in the protocol, B corresponds to (3), C to (5), D to (2), E to (4) and F to (6). Here again, A, which is P.A. No. 2, shows less necrosis than D, which is P.A. No.3. P.A. No. 2 is the P.A. serum which delayed death in white mice to 40 hours instead of reacting within sixteen hours as the majority did.

These guinea pigs received a total of .15cc to .2cc of toxin intradermally in these skin tests, and all developed marked gastro-intestinal symptoms within a week, terminating fatally within three to five weeks. Marked emaciation, indifference, roughened fur, accompanied by loss of appetite and in some cases enlarged ulcerated areas at the site of inoculations were the symptoms. Autopsy revealed no definite lesions, blood pictures were apparently normal, but marked distension of the abdominal cavity occurred. These deaths were attributed to the toxin, however, since other guinea pigs in the same cages remained well until inoculated in the same way.

Although the above experiments show that the sera of pernicious anemia and normal individuals did not completely protect white mice from lethal doses of welchii toxin, some variation was found in two cases of pernicious anemia sera, and one of normal serum which delayed death in white mice and also showed a corresponding protection in guinea pig skin tests.

Immune sera have been prepared by Patterson and Kast, and also by Bull and Pritchett. In both cases, definite protection was secured against infections with welchii bacilli. Patterson and Kast suggest that the human body acquires an immunity for its intestinal welchii and that in the case of pernicious anemia other factors, still obscure, exist which contribute to breaking down this immunity in order to permit the products of welchii to become effective.

Barach and Draper experimented on eight cases of pernicious anemia and nine normal individuals and were unable to demonstrate an anti-hemotoxin for welchii in their sera.

-II-

A COMPARISON OF THE HEMALYTIC PROPERTIES OF SIX FILTRATES OF A. WELCHII AND THE RESISTANCE OF PERNICIOUS ANEMIA AND NORMAL ERYTHROCYTES TO HEMALYSIS BY THESE FILTRATES.

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Several attempts were made to produce a toxic filtrate which could be used in the first part of this paper. However, the toxicity varied to such a degree that it was impossible to use them and they were reserved for these hemalytic experiments.

Filtrate No.1 was made from a stock culture. Liver infusion media, Ph 7.4 was inoculated and incubated 18 hours at 37 degrees centigrade under anaerobic conditions. It was then centrifugalized and the supernatant fluid passed through a sterile Berkfeld filter. When examined for potency it was found to produce only slight symptoms in two pigeons.

Filtrate No. 2. was also made from the stock culture. This time a casein digest media, made according to Kahn and Torrey, was inoculated under anaerobic conditions. The filtrate was found to be slightly toxic in larger amounts. The stock culture was discarded as a source for toxic filtrates.

The third filtrate was made from a strain of

Welchii isolated from the feces of a pernicious anemia case in the following manner: Fecal matter was diluted with sterile saline and passed through a sterile cheese cloth filter. The filtrate was heated to 80-85 degrees centigrade for fifteen minutes to eliminate all organisms except spore producers. Sterile milk was inoculated to determine the presence of welchii by the stormy fermentation. Cultures were made in deep dextrose agar for purification. Colonies picked from these cultures were inoculated into dextrose, lactose, saccharose and mannite fermentation tubes, and into milk and brain media for confirmation. All cultivation was carried out anaerobically in a navy jar and under sterile oil or vaseline which had a high melting point. After isolation, the organism was cultivated in brain media, from which the special media were inoculated.

The casein digest broth was inoculated from an eighteen-hour brain media culture. After eighteen hours' cultivation in this media, it was centrifugalized and the supernatant fluid filtered through a sterile Berkfeld. The filtrate was tested for sterility and for potency. This filtrate killed a pigeon in 24 hours in 2.5cc amounts. However, .5cc produced grave symptoms in an 18-gram mouse. followed by recovery.

The fourth filtrate was made according to the methods used by the Hygienic Laboratory at Washington, in the following way: 2½cc of an 18-hour broth culture of the

strain isolated above was inoculated into the breast muscle of a normal pigeon. The pigeon died within twelve hours. Part of the disintegrated muscle was removed aseptically into another broth tube and incubated 18 hours anaerobically. This was passed through two more pigeons, and in each case sterile precautions were used and strict anaerobiosis observed in cultivation. The muscle from the last pigeon was inoculated directly into a special media made up as follows: 500 grams of beef heart were infused in 1 liter of water in the ice box over night. The next morning this infusion was heated in the Arnold for thirty minutes and ten per cent peptone and five-tenths per cent NaCl were added. To each 300cc of this mixture, 200 grams of finely-cut veal were added. .2% glucose was sterilized in the autoclave separately and added after the final sterilization of mediae. A trial titration for final adjustment to Ph 7.4 was made on one 100cc of the media. The amount of NaOH necessary was then added to the other media before final sterilization in the autoclave. Sterile vaseline of high melting point was inoculated over the surface after the muscle had been inserted, and incubation again carried out for eighteen hours at 37 degrees centigrade. The broth was then siphoned into sterile tubes, centrifugalized and filtered through a sterile Berkfeld filter. 1½cc of this filtrate killed a pigeon in less than 12 hours,

and when titered in mice it was found that .12cc contained an M. L. D. for an 18-gram white mouse. About fourteen days elapsed before a pernicious anemia case was available to carry out any of the experiments in the first part of this paper, and when the filtrate was again titered for toxicity it was found that .5cc produced grave symptoms but not death.

A fifth attempt was made reproducing the technique above with practically the same results. In this case the M.L.D. was as low as .1cc when first titered, but in ten days had exceeded .35cc. These filtrates were too unstable to be used in the foregoing experiments, but were used in the following hemolytic experiments.

The same pernicious anemia case could not be secured for each filtrate, and so pernicious anemia No. 1 was used for filtrates 1, 2, 3, and 52. The same normal bloods were not used each time either. The following protocol shows the dilutions used and the method of setting up the mixtures:

PROTOCOL VI

Tube	Toxin Dilution	Amount	Saline	5% cells Amount	Source
1	1:10	2cc	0	.5cc	P.A.
2	1:10	"	0	"	N.
3	1:25	"	0	"	P.A.
4	1:25	"	0	"	N.
5	1:50	"	0	"	P.A.
6	1:50	"	0	"	N.
7	1:75	"	0	"	P.A.
8	1:75	"	0	"	N.
9	1:100	"	0	"	P.A.
10	1:100	"	0	"	N.
11	1:150	"	0	"	P.A.
12	1:150	"	0	"	N.
13	1:200	"	0	"	P.A.
14	1:200	"	0	"	N.
15	1:250	"	0	"	P.A.
16	1:250	"	0	"	N.
17	0	0	2cc	"	P.A.
18	0	0	2cc	"	N.

Readings made in thirty minutes and hour intervals were recorded. It was found that readings delayed over night did not give definite, clear-cut results.

The following table gives a record of the results. N.H. indicates no hemolysis; I.H. indicates initial hemolysis; S.H. indicates strong hemolysis; and C.H. indicates complete hemolysis. Only those dilutions in which hemolysis was found are listed in the first table.

		Thirty Minutes			Six Hours						
		Toxin Dilutions			Toxin Dilutions						
Filtrate	Source Blood	1:10	1:25	1:50	1:25	1:50	1:75	1:100	1:150	1:200	1:250
1	P. A.	S.H.	I.H.	N.H.	S.H.	S.H.	I.H.	N.H.	N.H.	N.H.	N.H.
	Normal	I.H.	N.H.	N.H.	S.H.	S.H.	N.H.	N.H.	N.H.	N.H.	N.H.
2	P. A.	S.H.	I.H.	N.H.	S.H.	I.H.	N.H.	N.H.	N.H.	N.H.	N.H.
	Normal	I.H.	N.H.	N.H.	S.H.	I.H.	N.H.	N.H.	N.H.	N.H.	N.H.
3	P. A.	S.H.	I.H.	N.H.	S.H.	S.H.	S.H.	N.H.	N.H.	N.H.	N.H.
	Normal	S.H.	I.H.	N.H.	S.H.	S.H.	I.H.	N.H.	N.H.	N.H.	N.H.
4	P. A.	S.H.	S.H.	I.H.	C.H.	S.H.	S.H.	S.H.	I.H.	N.H.	N.H.
	Normal	S.H.	S.H.	I.H.	C.H.	S.H.	S.H.	I.H.	N.H.	N.H.	N.H.
5	P. A.	S.H.	S.H.	I.H.	C.H.	S.H.	S.H.	I.H.	N.H.	N.H.	N.H.
	Normal	S.H.	S.H.	I.H.	C.H.	S.H.	S.H.	I.H.	N.H.	N.H.	N.H.
52	P. A.	S.H.	S.H.	I.H.	C.H.	C.H.	S.H.	S.H.	S.H.	I.H.	N.H.
	Normal	S.H.	S.H.	I.H.	C.H.	C.H.	S.H.	S.H.	I.H.	N.H.	N.H.

The filtrates are numbered according to the descriptions given above of their production. No. 52 is the toxin obtained from H. K. Mulford.

Only a slight variation is apparent in this table in the resistance of the pernicious anemia cells and normal cells to hemolysis by these filtrates. Wherever any variation occurs the normal cells show more resistance, however. The thirty minute readings showed that the pernicious anemia cells succumbed a little faster to hemolysis than did the normal cells.

A greater variation occurred in the resistance to the different filtrates. Those produced from stock cultures hemolyzed the cells in higher concentrations than did the filtrate produced from the strain of welchii isolated from the feces of the pernicious anemia case. Filtrates 4 and 5 showed hemolytic qualities comparable to #52. The pernicious anemia cells showed a greater susceptibility to #52 than did the normal cells.

After investigating the toxic and hemolytic qualities of welchii filtrates, Patterson and Kast[21] divided them into four groups. Group I possessed both hemalytic and toxic qualities. Group II was toxic but not hemolytic. Group III was hemolytic but not toxic. Group IV was non-hemolytic and non-toxic. According to their classification, filtrates 1, 2 and 3 above belong to group III, showing some hemolytic properties, but negative toxic properties. Filtrates 4 and 5 would belong to group I during the first week after their production, but due to the instability of their toxic property, they would then be classified in group III. #52 is undoubtedly in group I.

-III-

THE RESISTANCE OF ERYTHROCYTES TO PHYSICAL TRAUMA STUDIED.

The resistance of red cells to various agents such as acids, other chemicals and hypotonic solutions have been investigated under varying conditions. No record of any observations of red cells under physical trauma could be found, however, and Dr. N. P. Sherwood suggested that such an investigation be carried out. He devised the apparatus to be used, which is shown in the accompanying photograph.

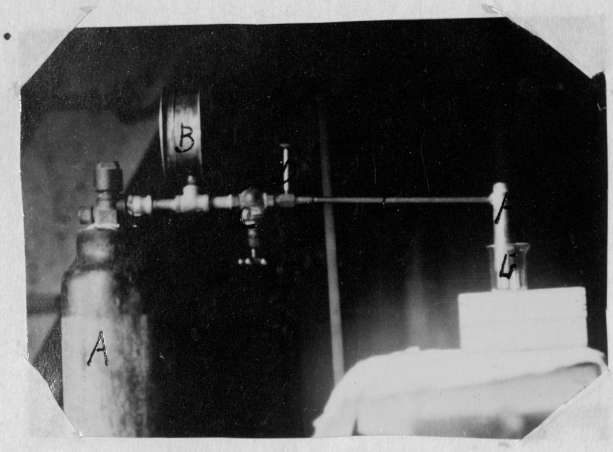

Figure IV.

The tank, A, was filled with CO_2 for the first set of experiments and was later refilled with air under pressure. B is a pressure guage registering 100 pounds, C the cock to release the pressure, D the tube through which the blood was introduced into the carrier E, F the inverted brass tube against which the pressure forced the cells to produce trauma. G is a beaker in which the blood was collected after being forced out by pressure.

A variety of methods were employed at first to determine the best conditions under which the work might be carried out. Dilutions of blood were varied from 2% to 10% of the volume of the blood to be used, pressures were varied and the methods for cleaning the carrier and the brass tube between blowings were investigated. These resulted in the following technique, which was carefully employed in all recorded cases. After serum was removed from the cells the cells were washed twice in normal saline and a 2% suspension made. This suspension gave much better microscopic results than did the higher concentrations. Three cc's of this suspension were introduced into D and the cap screwed down. The pressures used were 25 pounds, 50 pounds, 75 pounds and 100 pounds. The amount to be used was released at C and the pressure forced the cells against F from which they dropped into the beaker. The carrier and tube were rinsed in water, dried on a hot radiator, and cooled to room temperature before being used again. For the microscopic examinations for hemolysis and agglutination small, clean precipitin tubes were used. These were set up in a series of five for the varying pressures, and a control. A capillary pipette was used to transfer from the beaker to the tubes. After each blowing, a drop of blood was placed under the oil immersion for further destruction which of course was not evident in the microscopic examination.

To determine the resistance to hemolysis records were made after about one hour and eighteen hours, or over night. A series of twenty-five normals were blown from CO_2 under pressure. At this time only one pernicious anemia specimen was available. It was used three different times with quite consistent results. Twenty-four normals were obtained for the series blown under air pressure. At this time it was possible to get nine pernicious anemia cases.

Agglutination was found to be almost constantly associated with hemolysis so it was recorded too. The table below gives a summary of the percentages of normals and pernicious anemias, which showed initial hemolysis and initial agglutination at varying pressures with CO_2 and air.

	Initial Hemolysis				Initial Agglutination			
	CO_2		Air		CO2		Air	
Pressure	Normal	P.A.	Normal	P.A.	Normal	P.A.	Normal	P.A.
25 lbs	.04%	--	33%	30%	94%	33%	94%	45%
50 lbs	62½%	100%	66%	60%	6%	66%	--	55%
75 lbs	29%	--	--	10%	--	--	--	--
100 lbs	.04%	--	.05%	--	--	--	--	--
Cont.	--	--	---	--	--	--	---	--

The largest percentage of initial hemolysis occurred at fifty pounds pressure with both CO_2 and air. With CO_2, 29% of the normal cells showed hemolysis at 75 pounds pressure, and .04% at 100 pounds. Due to the lack in number of P. A. used

in this series it is hardly possible to draw any comparison between the resistance of pernicious anemia and normal to CO_2 pressures. However, this case reacted identically each of the three times it was used.

The normal cells resisted CO_2 better than they did air under pressure, whereas 10% of the pernicious anemia cases did not hemolyze below 75 pounds pressure.

Agglutination also occurred very regularly, although it occurred at lower pressures. In the table above, the normal cells showed agglutination almost constantly at 25 pounds, whereas the pernicious anemia cells showed the highest percentage of initial agglutination at 50 pounds. This would indicate a slightly higher resistance of pernicious anemia cells to agglutination than normal cells.

Microscopic examinations were made to observe any abnormality in individual cells. Records were made of agglutination, distortion, swelling of cells, crenation, fragmentation and any great amount of disintegration, poikilocytosis and anisocytosis. The following tables give a summary of the per cent of cases which gave positive reactions for each item recorded at the varying pressures.

AIR

Pressure	Agglut'n		Distr-tion		Swelling		Brenation		Fragm't-ation		Poikilocytosis		Anisocytosis	
	N	PA	N	PA	N	PA	N	PA	N	PA	N	PA	N	PA
25#	55	44	55	100	66	66	33	11	16	88	0	100	0	100
50#	22	77	83	100	72	77	50	44	38	100	0	100	0	100
75#	38	66	84	100	88	88	50	55	66	100	0	100	0	100
100#	16	55	88	100	88	88	38	22	83	100	0	100	0	100
Cont.	0	0	0	0	0	0	66	11	0	0	0	100	0	100

CO_2

25#	32	0	56	100	32	100	40	66	20	100	0	100	0	100
50#	40	66	68	100	44	100	40	100	24	100	0	100	0	100
75#	32	33	64	100	36	100	40	100	20	100	0	100	0	100
100#	24	0	56	100	44	100	30	100	20	100	0	100	0	100
Cont.	0	0	0	0	0	0	50	100	0	0	0	100	0	100

(Numerals indicate pernectages)

In examining these tables the high percentages of pernicious anemia cells show positive reactions at almost every pressure indicate an increase in fragility over normal cells. The percentages mean the number of cases in which these reactions were found, a great variation in degree of reaction was encountered throughout.

Some slides showed only a few cells which were distorted in the entire drop. Whereas others contained fields with several distorted cells in one field.

By distortion was meant those cells which did not have the characteristic biconcave appearance, but appeared to have had the centers blown out to make a deep hollow concavity on one side. Other cells appeared twisted, some looked like dumb-bells. Some were bent to form an arc. In the pernicious anemia cases careful comparisons were made with controls in order not to call characteristic pernicious anemia cells distorted. Some duplication may have arisen, but in each case the distortion was very much more marked in the blown slides than in the controls.

The swollen appearance of cells was found quite regularly. These cells had a spherical shape instead of being biconcave.

Crenation often occurred in controls, and it was interesting to note that in a good many cases where the controls were apparently 100% crenated, after having been blown only a few cells in the slide showed crenation. Normal cells showed greater amount of irritation than the pernicious anemiacells as indicated by the higher percentages found in which crenation occurred.

Fragmentation consisted of a breaking up of cells. This occurred in almost every pernicious anemia case at nearly all pressures, whereas the percentage of normals increased as the pressure was increased. Here again the degree varied to a marked extent. Fragmentation was in-

dicated by cells which apparently had small pieces chipped out of the edges, in some cases the cells were cracked and apparently pulled apart, although not entirely severed.

Poikilocytosis and anisocytosis were found in all cases of pernicious anemia and in none of the normals. The degree of poikilocytosis increased under pressure.

To determine whether the distortion might be temporary, several slides were sealed with vaseline and the cells examined after one hour, three hours and eight hours. In every case no change could be detected in distortion. Occasionally the swollen cells seem to become crenated, but as a rule they remain constant. The condition is apparently permanent.

SUMMARY AND CONCLUSIONS

1. No protection could be demonstrated in the sera of six normal and eight pernicious anemia individuals against a lethal dose of welchii toxin for 18-gram white mice.

2. Death was delayed in white mice, which received lethal doses of toxin by two pernicious anemia sera. One case was delayed eight hours, the other twenty-four hours. One normal serum delayed death three hours over the maximum time. This case would hardly have attracted attention if a corresponding parallelism had not been found in the guinea pig skin tests.

3. The pernicious anemia serum which delayed death twenty-four hours in white mice also showed a comparatively less reaction in skin tests on guinea pigs. The normal serum mentioned above, when used in varying concentrations with toxin in guinea pig skin tests, showed less ulceration in the higher concentrations of serum.

4. The small amount of toxin used in these skin tests (not exceeding .2cc) caused death in all the guinea pigs in from two to five weeks accompanied by marked gastro-intestinal symptoms.

5. In studying the hemolytic properties of five filtrates produced from two different strains of Cl. welchii and one toxin obtained from Mulford's, considerable variation

was found in the dilutions at which hemolysis occurred. The Mulford toxin had the greatest hemolytic property, the filtrates from the strain isolated from pernicious anemia feces had a much greater hemolytic property than filtrates produced from a stock culture.

6. Some variation occurred in the comparative resistance of normal and pernicious anemia cells to hemolysis by these filtrates, the normal cells showing slightly greater resistance.

7. In examining erythrocytes for their resistance to physical trauma it was found that both normal and pernicious anemia cells were hemolyzed most often at 50 pounds both in the case of air under pressure and CO_2 under pressure. The greatest variation from this was 29% of normal cells which showed initial hemolysis at 75 pounds when blown with CO_2 under pressure. The normals were more resistant to CO_2 under pressure than they were to air under pressure. Only one pernicious anemia case showed initial hemolysis at 75 pounds, this was blown under air pressure. It would indicate that the pernicious anemia cells had a greater resistance to air pressure than normal cells.

8. Macroscopic examination for agglutination after physical trauma occurred quite constantly at 25 pounds in the case of normal cells, both blown under air and CO_2 pressures. Pernicious anemia cells agglutinated in larger percentages at 50 pounds. Agglutination occurred very consist-

ently with hemolysis throughout all these experiments.

9. The microscopic examinations of cells after having been blown under pressure, revealed 1) a marked succeptibility of pernicious anemia cells to distortion and fragmentation under air pressure. The same results were obtained in CO_2 experiments, although only one pernicious anemia individual was used--repeating the case three times. 2) the normal cells showed an increase in susceptibility as the pressures were increased, which is shown by the increased perdentages in distortion and fragmentation especially. 3) These distortions were permanent. Upon examination after several hours' standing, slides still showed the same degree of distortion and fragmentation.

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