BLOOD CHEMICAL CHANGES IN EXPERIMENTAL STREPTOCOCCUS SEPTICEMIA

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Studies in the chemical changes in the blood of animals experimentally infected with virulent *Streptococcus hemolyticus* have seldom been made. Dragstedt (1) determined the actual pH of the blood of four rabbits which had been given large injections (2 to 4 cc.) of cultures of this organism intraperitoneally. In the two animals which died the pH fell from 7.7 and 7.6 to 7.5 and 7.3 respectively. The animals which recovered showed actual increases in acidity to the same degree, leading one to suppose that this factor could not have been of outstanding importance as a cause of death. Hirsch (2) injected various types of bacteria, including a hemolytic streptococcus, into rabbits by the intravenous route. With this microorganism he found a lowering of the CO_2 capacity to 48 volumes per cent after 5 hours, followed at 24 hours by an alkalosis (80 volumes per cent) and normal figures throughout the following 6 days. It is not stated whether the streptococcus infection was fatal for the rabbit. In a later communication (3) he again reported the results of a similar injection into a rabbit. Here the CO_2 capacity fell from a preliminary figure of 59 volumes per cent to 54 volumes at 5 hours, and remained normal thereafter. The blood sugar concentration of this animal was increased, rising from 106 mg. at the time of infection to 135 mg. at 5 hours, 120 mg. at 23 hours, and 141 mg. at 29 hours. The streptococcus used is referred to as pathogenic. The data show, however, that the constituents studied were not much affected by the organism used.

The purpose of the work reported here was to ascertain the cause of death from streptococcus septicemia in rabbits, so far as it may be related to chemical changes. To this end the following constituents were studied: Blood sugar, liver glycogen, CO_2-combining capacity, inorganic phosphorus, calcium, non-protein nitrogen, and creatinine.

Material and Methods

The carbon dioxide-combining capacity and the creatinine were determined by the usual laboratory methods. For the other constituents the following techniques were employed: blood sugar (Folin's micro method (4)); glycogen (Slosse

(5)); inorganic phosphorus (Tisdall (6)); calcium (the Clark-Collip modification of the Kramer-Tisdall method (7)); non-protein nitrogen (Folin and Svedberg (8)).

Rabbits were used. Doses of a 24 hour broth culture of *Streptococcus hemolyticus*, varying in amount from 0.3 to 0.75 cc., were given in the ear vein. This organism is highly virulent for rabbits when given intrapleurally, as little as one ten-millionth of a cubic centimeter of a culture producing a rapidly fatal empyema. The same strain given intravenously is much less virulent, probably because when given by this route the organisms are brought directly in contact with tissues capable of destroying them. Hopkins and Parker (9), using a streptococcus virulent for rabbits, have shown that the lung and liver are particularly active in this destruction, and that only those microorganisms which lodge in the muscles continue to multiply. From the muscles they are washed out into the blood stream to give rise to the septicemia.

A total of seventeen animals was used in the experiments. They fall into two groups, depending upon the length of survival after infection. Those which died within 2 to 4 days are designated as having fulminating infections. The average length of survival in this group was 61 hours and the average dose 0.5 cc. Ten of the animals are included in this series. In the second group, which are designated as acute cases, the survival times averaged 6 days and the dose averaged 0.4 cc. The remaining seven animals are in this category. It is clear that the relationship between size of dose and survival time is by no means absolute.

About 12 cc. of whole blood are necessary to complete the tests named above. As it would be injurious to the animal to take this amount of blood at short intervals during the course of a severe infection, not all the tests were done on any one animal at a given time. As a result it is necessary to present the data as averages.

RESULTS

The results of the determinations are given in Figs. 1 to 4, and the averages upon which the curves are based are shown in Table I. The abscissae represent fifths of the period during which the disease ran its course. In this way the acute type of infection is foreshortened in relation to the fulminating type, but it was thought that any disadvantage arising from this method of representation would be compensated for by the greater ease in comparing the two curves.

Blood Sugar.—Fig. 1. The averages for this constituent show that in the fulminating group the blood sugar drops at a rather constant rate throughout the infection. In the group of acutely infected animals the drop does not begin until the disease has run about two-fifths of its course, but from that time on the glucose gradually lessens. Nevertheless, the levels reached in the terminal stages by both groups are not those at which a hypoglycemia can be considered present.

During the last part of the disease the rabbits do not eat, and it was considered possible that part of the fall in blood sugar might be accounted for in this way. The literature on the effect of starvation on rabbit blood glucose shows, however, that a curve such as this is not obtained in animals from which food is being withheld. On the contrary a constant level of blood sugar is maintained for several days,

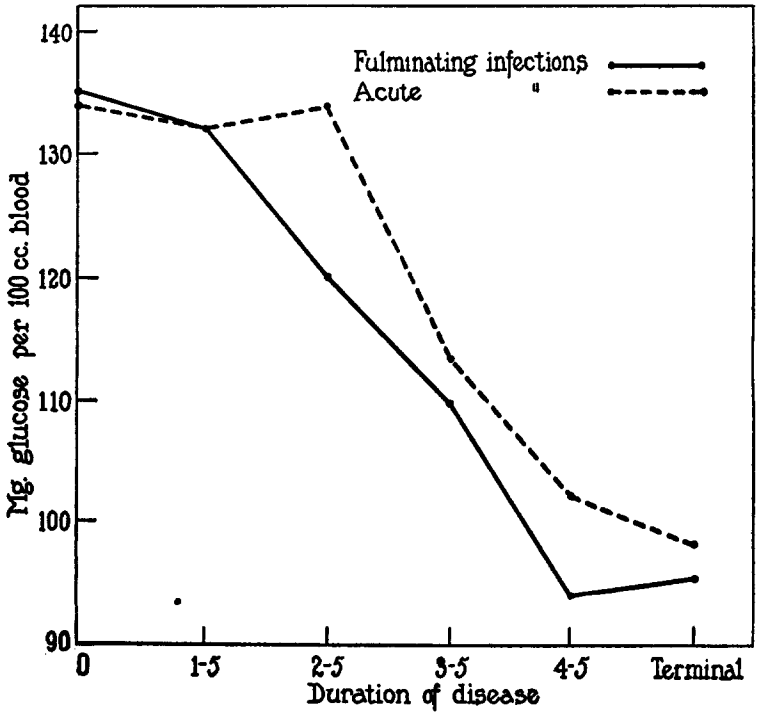

FIG. 1. Average concentration of blood sugar in the blood of rabbits with acute and fulminating infections with *Streptococcus hemolyticus*.

and the levels instead of decreasing steadily as in the streptococcus infected rabbits show increases and decreases throughout the period (Kisch, Simons, and Weyl (10)).

Liver Glycogen.—In six animals, two of the fulminating and four of the acute type, the liver glycogen was determined at death. In no case was glycogen absent, and expressed as glucose, it varied from 1 to 21.6 mg. per gram of liver, averaging 8.1 mg. for the series. Such a

result might of course be expected in view of the nearly normal levels of blood glucose at death, although it has been shown (Linton (11)) that in acute trypanosome infections in rats the liver glycogen disappears at a relatively long period before death, while the blood sugar remains at a normal level until the agonal stage, when a severe hypoglycemia is present.

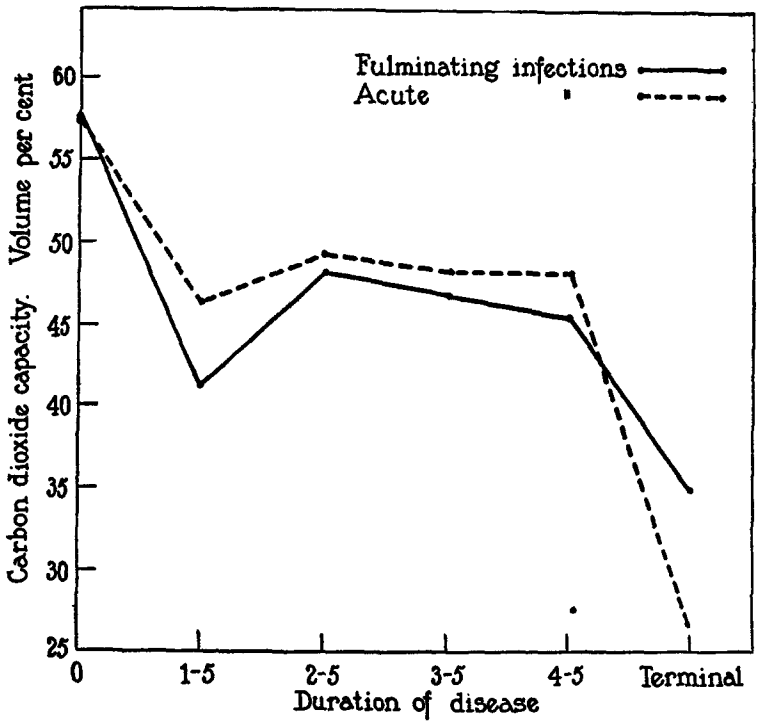

FIG. 2. Average carbon dioxide capacity in rabbits with acute and fulminating infections with *Streptococcus hemolyticus*.

Carbon Dioxide-Combining Capacity.—The curves of the CO_2 capacity are given in Fig. 2. In both groups an immediate drop occurs, to 46.0 volumes in the acute and to 41.5 in the fulminating cases, followed by a rise which brings the capacity back to the lower limit of the normal range of variation in the rabbit. This value persists through the remainder of the disease at a rather constant level, until the terminal stage is reached, when a second sharp

drop occurs. In the acutely infected animals the final value is 26.2 volumes and in the fuminating infections it averages 34.8 volumes per cent.

Inorganic Phosphorus and Calcium.—The data for these constituents are presented together in Fig. 3, since it seems well established that there is a reciprocal relation between them. The inorganic phosphorus

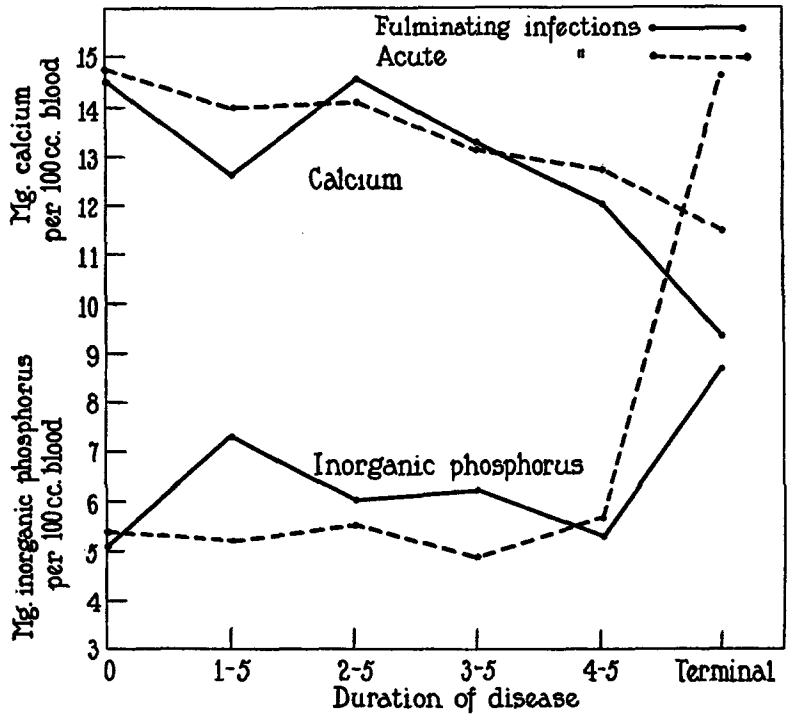

FIG. 3. Average concentrations of calcium and inorganic phosphorus in the blood of rabbits with acute and fulminating infections with *Streptococcus hemolyticus*.

in the acute cases shows a marked terminal rise, reaching an average level of 14.2 mg. per 100 cc. A similar terminal rise, although to a less marked degree, is shown in the fulminating infections. Here the average figure (8.6 mg.) approximates the upper limit of the normal variations (Harnes (12)).

The study of the calcium variations was undertaken because of the

symptoms of disturbance of concentration of this element. These include weakness of the limbs during the terminal portions of the disease and a considerable degree of hyperexcitability. The average figure for the fulminating group, 9.3 mg., shows a more severe change in this constituent in this group than in the acutely infected rabbits, where the terminal figure is 11.5 mg. While the general trend of the

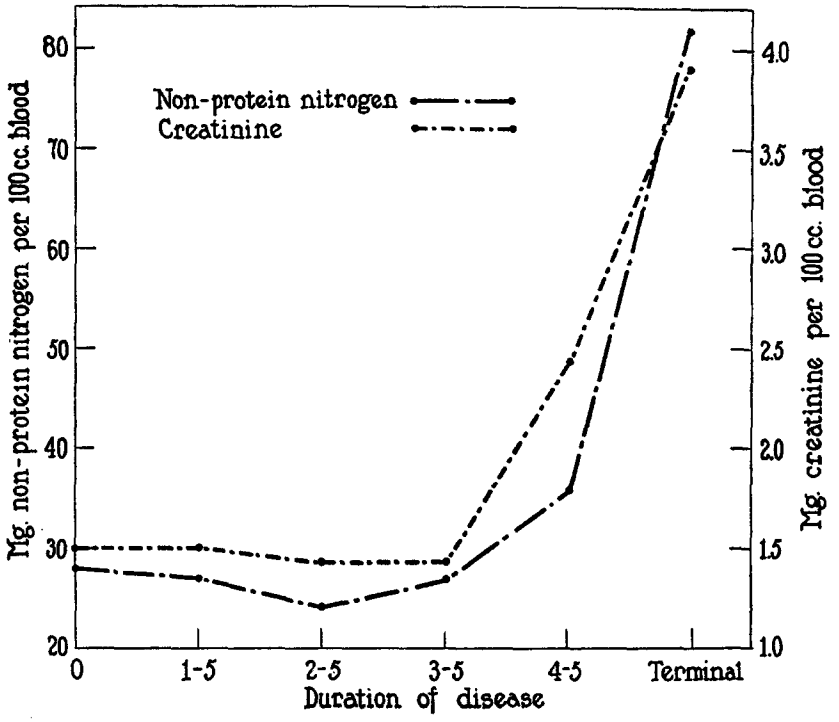

FIG. 4. Average concentrations of non-protein nitrogen and creatinine in the blood of rabbits infected with *Streptococcus hemolyticus*. Combined data from fulminating and acute cases.

curves is downward, it can only be concluded that the drop in calcium is a terminal phenomenon.

Non-Protein Nitrogen and Creatinine.—These two constituents were determined as measures of kidney function (Fig. 4). As the curves show they remain at normal concentrations in the blood until late in the disease, at which time they show marked rises, reaching

an average of 81.6 mg. for the non-protein nitrogen, and 3.9 mg. for the creatinine. The data for the fulminating and acute cases are combined in the chart, and given separately in Table I.

Histological Changes.—In sections of kidney taken from animals killed before the disease had reached its terminal stage (as shown by non-protein nitrogen and creatinine determinations), the histological changes were slight and consisted essentially of cloudy swelling of the

TABLE I

Changes in Averages of Blood Constituents in Fulminating and Acute Septicemias Due to Streptococcus hemolyticus

Duration of disease	Blood sugar		CO_2 capacity		Inorganic phosphorus	
	Fulminating	Acute	Fulminating	Acute	Fulminating	Acute
0	135	134	55.5	55.2	5.1	5.4
1–5	132	132	41.5	46.0	7.2	5.2
2–5	120	134	48.3	48.9	6.0	5.6
3–5	110	117	47.3	48.3	6.2	4.9
4–5	94	111	43.9	48.3	5.2	5.6
Terminal	95	101	34.8	26.2	8.6	14.2
Duration of disease	Calcium		Non-protein nitrogen		Creatinine	
	Fulminating	Acute	Fulminating	Acute	Fulminating	Acute
0	14.5	14.7	28.1	27.1	1.4	1.8
1–5	12.6	13.9	32.5	20.8	1.5	—
2–5	14.4	14.0	28.0	20.7	—	1.4
3–5	13.3	13.0	23.9	27.5	—	1.4
4–5	12.0	12.7	27.2	37.1	4.2	1.5
Terminal	9.3	11.5	61.9	96.3	4.2	3.5

tubular epithelium. The glomeruli were normal. Kidneys taken at death, after the alkaline reserve had been exhausted, showed, on the other hand, a severe nephritis. The tubular epithelium was severely injured, the tissue was edematous and hemorrhagic, and the glomeruli were destroyed in part. It is evident that the severe histological changes are terminal and can be explained by the occurrence of an actual acidity of the blood. The histological changes are more severe in animals with acute infections than in those in which death occurs

in a briefer period. This difference may be due to the longer continuation of the stage of uncompensated acidosis.

DISCUSSION

It is evident that the results obtained may be explained on the assumption of a large amount of acid production *in vivo* by the streptococcus. The buffers of the blood respond to the introduction of the acid, at first by a relatively great drop, followed by the maintenance of a constant level. The addition of more and more acid does not change the reaction of the blood, nor the CO_2 capacity. Finally, however, the buffer is exhausted and the addition of still more acid by the constantly increasing numbers of organisms brings on a state of uncompensated acidosis.

This final drop in the buffer, and the accumulation of acid resulting therefrom, causes a severe injury to the kidneys, which shows itself in the accumulation in the blood of non-protein nitrogen and creatinine. The inorganic phosphorus content also increases for the same reason, and as a result the calcium falls to abnormally low levels.

The fall in blood sugar may be accounted for by an actual direct use of glucose by the streptococcus, or indirectly by an injurious effect of the bacteria upon the liver. It is probable that both factors unite to produce the observed result. Further experiments are now in progress to determine more closely the cause of the lowering of the blood glucose.

The immediate cause of death in this condition appears to be the severe changes occurring in the kidneys, changes which result from the production of acid by the microorganisms.

SUMMARY

The following changes have been found to occur in rabbits given fatal intravenous doses of *Streptococcus hemolyticus*:

The blood sugar concentration drops at a constant rate throughout the disease, but does not reach a condition of hypoglycemia.

Glycogen is present in the liver at death.

The CO_2 capacity is lowered markedly at first, then returns to a somewhat higher level, at which it continues until the terminal stage of the disease, when the acidosis becomes very marked.

Inorganic phosphorus is markedly increased in concentration at the terminus of the disease. This increase is greater in animals showing an acute course than in those in which the disease is of the fulminating type.

Calcium also shows terminal changes, decreases occurring in both groups. In the acutely infected rabbits the decrease is less than in the fulminating group, although in both a pathological level is reached. Non-protein nitrogen and creatinine are greatly increased in the terminal stages, in both groups of animals.

It is suggested that these observations can be explained on the assumption of a large amount of acid production by the streptococcus *in vivo*.

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