A COMPARATIVE STUDY OF LYMPH AND SERUM FERMENTS DURING PROTEIN SHOCK REACTIONS.

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During the course of investigations concerning the mechanism of recovery following the so called "protein shock therapy" we have been interested in determining the comparative changes that occur in the lymph and blood serum and the possible manner in which these changes are brought about.

It is well known that certain substances, which Heidenhain (1) classified as lymphagogues of the first class, among them peptone, egg albumin, tissue extracts, etc., cause a marked increase in the lymph flow, supposed to be derived largely from the liver. This increased flow may continue a considerable time following such injection. Teague and McWilliams (2) have recently advanced the interesting explanation that this phenomenon is responsible for the therapeutic effect of the protein shock in that the antibodies of the blood are forced into the lymph spaces and there destroy the invading organisms. There is no doubt that the bacterial injections used in these experiments (Bacillus coli) do bring about a great augmentation of the lymph flow, as will be observed from the charts, and the explanation of Teague and McWilliams may well account in a large measure for the therapeutic effects.

EXPERIMENTAL.

The technique used has been identical with that described in the previous papers. Dogs of from 20 to 40 kilos were anesthetized, a thoracic duct fistula was established, and after recovery from the operation killed colon bacilli were injected intravenously, the amount used for the shock varying from two to three slants of a 24 hour
agar culture. When necessary small doses of morphine were given, although we have endeavored to avoid such measures. If the animals are injected too soon after the operation and before complete recovery has been made from the anesthetic, considerable resistance to the shock may be manifest and the temperature reaction delayed for several hours.

Lymph Volume.—The increase in the rate of flow of the lymph follows immediately upon the injection (Text-fig. 1) and in severe intoxications two periods of maximum flow seem to occur, the first immediately after the injection and persisting for from 20 to 30 minutes, the second after approximately 1 hour, the latter increase being continued over a longer period of time. When the intoxication is not so great the two phase curve does not occur, the increase being less in extent but persisting for a longer period of time.

Concentration of the Lymph and Serum and the Relation to the Stalagmometric Determination.—The concentration of the lymph following the injection is considerably increased, as determined by the
Kjeldahl method for total protein nitrogen, although the concentration of the non-protein nitrogen may decrease, as shown in Table I.

<table>
<thead>
<tr>
<th>Time of sample</th>
<th>Blood</th>
<th>Lymph</th>
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<tbody>
<tr>
<td></td>
<td>Total nitrogen per cc.</td>
<td>Non-protein nitrogen per cc.</td>
</tr>
<tr>
<td>a.m.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>11.30-12.00 (before injection)</td>
<td>9.8</td>
<td>0.45</td>
</tr>
<tr>
<td>12 n. 2 slants of colon bacilli injected.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.m.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>12.05-12.15</td>
<td>9.5</td>
<td>0.50</td>
</tr>
<tr>
<td>12.15-12.30</td>
<td>9.7</td>
<td>0.45</td>
</tr>
<tr>
<td>12.30-1.00</td>
<td>9.8</td>
<td>0.50</td>
</tr>
<tr>
<td>1.00-1.30</td>
<td>9.8</td>
<td>0.50</td>
</tr>
<tr>
<td>1.30-2.00</td>
<td>9.5</td>
<td>0.50</td>
</tr>
<tr>
<td>2.00-2.30</td>
<td>9.5</td>
<td>0.50</td>
</tr>
</tbody>
</table>

The effect of the concentration of the lymph is observed directly in the rate of the stalagmometric flow, which is decreased when the concentration becomes greater. This does not always hold true for the blood serum, however, as shown in Text-fig. 2. A considerable diminution of the number of drops per minute was observed, without a corresponding increase in the concentration of the serum. Such a change must be due to an alteration in the dispersion of the serum colloids.

Antiferment.—In a preceding paper (3) it was shown that considerable fluctuations are to be observed in the antiferment titer of the lymph following feeding. The changes noted following protein shock make it probable that the antiferment is supplied to the blood stream wholly by way of the lymphatics, and not directly from the cells to the blood. In Text-fig. 3, A and B, are illustrated two curves of the antiferment index (0.05 cc. of serum and lymph) following intravenous bacterial injection.

In Text-fig. 3, A will be observed the prompt and marked rise in the antiferment of the lymph stream while the titer of the serum remains unaltered. Text-fig. 3, B shows a more gradual increase in the antiferment of the lymph with a coincident fall in the titer of
the blood serum. The increase in the antiferment observed in the lymph persisted in our experiments for the duration of the time under observation, usually about 5 hours. The increase probably would continue if the animal were permitted to live for a longer period of time. No relation to the stalagmometric determination or to the protein concentration of the fluids was observed.

**Protease.**—The effect of the bacterial shock on the protease content of the lymph and serum is marked in extent. Three types of reaction may be distinguished: (a) the fluctuations in titer may
occur simultaneously, (b) those of the serum may precede those of the lymph, and finally, (c) there may be no relation of the one to the other, as illustrated in Text-fig. 4, C, in which the protease of the lymph increased and that of the serum diminished. Until more information concerning the origin and the distribution of these ferments in the vascular channels is available, it seems useless to endeavor to interpret these findings.

Text-Fig. 3. Antiferment of the lymph and serum following vaccine injections. A and B represent two different types of reaction.

**Peptidase.**—The fluctuations of the peptidase, or ereptase titer, do not parallel those of the protease; indeed the curves may be quite dissimilar. As a rule the increase makes its appearance later than that of the protease and is less extensive. When alterations in titer do occur they appear almost simultaneously in both the lymph and serum, although in a few experiments the ferment was first to be observed in the serum. It is at any rate apparent that the entrance into the blood stream can be direct and does not need to take place via the lymph channels, although under normal conditions, i.e., feeding, this seems to be one portal of entry.

**Lipase.**—While the increase in this ferment occurs in both lymph and blood following shock, it seems to make its appearance first in the serum (Text-fig. 5). In Text-fig. 5, A it will be observed that
while the lymph titer was greater than that of the blood at first, the increase was first apparent in the serum, although the final titer

Text-Fig. 4. Protease titer following vaccine injections. A, B, and C represent three different types of reaction.

was equal in both lymph and serum. In Text-fig. 5, B is illustrated an experiment in which the increase occurred primarily and to the greatest extent in the serum.
text-fig. 5. Lipase titer following vaccine injections expressed in cc. of 1/20 sodium hydroxide. A and B represent two different types of lipase reaction.

Diastase.—The diastatic activity of both the serum and lymph remained practically equal and without change following the bacterial injections in all the experiments.

Discussion and summary.

The relation of two phenomena involved in the mechanism of recovery following protein shock therapy is shown in these experiments to be due to changes that concern the lymph rather than the serum. The first of these, the increase in the rate of flow of the lymph, has been suggested by Teague and McWilliams as a possible factor in recovery from infection when due to bacteria proliferating in the lymph spaces and inaccessible to the antibodies of the serum (typhoid). By means of the protein shock, antibody-rich fluids (serum) are forced into the lymph channels. That the antibodies of the serum are augmented following the shock has been demonstrated by Culver (4). With this possibility in mind it is to be expected that bacterial infection not confined to the lymph spaces will not be influenced by shock therapy to the same extent. How far this holds true we are unable to state, although von Decastello (5) has called attention to the fact that while he was able to cause rapid lysis or a crisis in typhoid patients following the shock reaction, the injection of a similar amount of vaccine in typhus fever was without effect. The second factor, the great increase in the antiferment, is clearly due to the amount of the antiferment entering the general
circulation through the lymph stream. This accounts for the marked fluctuations observed in the titer of the serum antiferment in patients following protein shock. If, for instance, the original titer of the lymph is less than that of the blood, the first flushing of the lymphatic current into the blood channel will tend to lower the titer of the serum, but with the increased amount in the lymph after the shock the titer of the serum will also increase.

Bacteria proliferate best where the antiferment is absent, as Wright (6) has noted in his studies on war wounds, a fact that is also commonly applied in bacteriological technique when we employ ascitic fluids, containing relatively little antiferment, preferably to serum, in culture media.

The increased lymph flow that follows the shock reaction would have value then, not only in forcing specific antibodies into the lymph channels, but in increasing the antiferment there as well, which would aid in checking the growth of bacteria.

BIBLIOGRAPHY.