SEPARATION AND ASSAY OF LYSINS AND LYSIN-INHIBITOR COMPLEXES IN BLOOD AND TISSUES*

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The lytic substances found in extracts of tissues have been shown to exist as lysin-inhibitor complexes of relatively low hemolytic activity, from which the much more active alcohol-soluble lytic components can be extracted (Ponder, 1951; Tyler, 1949, 1950). Some of these lytic components are soluble in ether as well as in alcohol while others are alcohol-soluble but ether-insoluble, and it is likely that they are related to the ether-soluble and ether-insoluble lysins which have been described as obtainable from plasma and serum by alcohol extraction. These lysins, however, have been so differently described by different observers that the first step towards a simplification of the subject must be a standardization of method which will allow one to prepare the lysins reproducibly, both from tissues and from plasma, and to compare them with one another as regards activity.

This paper is concerned with three related aspects of this problem:—(1) the comparison of the lysins obtainable from blood with those obtainable from tissue such as mouse liver, (2) the application of paper chromatography and related techniques to the separation of these lysins and of their complexes, and (3) various observations on the nature of the lysins and on some of the properties of the inhibitors which form part of the lysin-inhibitor complexes.

I

The Relative Hemolytic Activity of Extracted Material

The essential steps of the various procedures which have been described for the extraction of lysins from blood and plasma can be combined into the following single process.

Methods for Extraction and Titration

Six ml. of blood, plasma, or washed cells with a volume concentration equal to that in the blood are added to 20 ml. of 95 per cent alcohol at 25°C. The blood, plasma, or washed cells may either be fresh or may have been preincubated at 37°C for various

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lengths of time. After standing for an hour or so, the material is filtered, the filtrate is divided into two equal portions, and both are evaporated to dryness in evaporating basins placed in a current of warm air (about 40°C).

Lysins insoluble in cold ether ("lysolecithin-like substances") are obtained from the residue of the first portion by dissolving it in 1 ml. of warm 95 per cent alcohol and then adding 10 ml. of ether. The preparation is kept at 4°C. for 12 hours. It is then centrifuged, and the supernatant ether is discarded. The precipitate is shaken up with 10 ml. of ether, and the preparation is again allowed to stand for 12 hours at 4°C. At the end of this time it is centrifuged, the supernatant ether is discarded, and 3 ml. of isotonic NaCl-buffer is added to the precipitate. Insoluble material is removed by centrifuging, and the clear supernatant fluid is dried in a current of warm air. The final step is to bring the dry residue into solution by adding 3 ml. of water, any material remaining undissolved being again removed by centrifuging. In this procedure all ether is removed from the final product, and alcohol-soluble material which becomes insoluble in saline after treatment with ether is also removed.

Ether- (and acetone-) soluble lysins are obtained from the residue of the second portion by adding 3 ml. of ether to it, mixing, and then adding 7 ml. of acetone. The material is filtered, and the filtrate is evaporated to dryness under the same conditions as before. The extraction with ether-acetone is repeated, and the filtrate is again evaporated to dryness. The residue is taken up in 3 ml. of 95 per cent alcohol and is transferred to a small Whatman diffusion shell supported on a glass tube open at both ends, by means of which it can be held at any desired level in a large vessel containing cold (4°C.) NaCl-buffer at pH 6.5. Diffusion is allowed to continue for 48 hours at 4°C. The level of the fluid in the shell should be the same as that in the vessel initially, but the level rises as diffusion proceeds, so that at the end of 48 hours the volume of the fluid in the shell is usually greater than 3 ml. The volume of the opalescent fluid found in the shell at the end of this time has therefore to be reduced to 3 ml. by evaporation in a current of warm air; this final evaporation (which produces a small tonicity change of minor significance) is conveniently carried out in beakers of 5 ml. capacity, marked at 3 ml.

The hemolytic activity of the final preparations of ether-insoluble and of ether-soluble lytic material is determined by measuring the time required for the production of complete hemolysis, at 37°C., in a system containing 0.5 ml. of the final preparation and 0.1 ml. of a suspension made by suspending the thrice washed red cells of 1 ml. of human blood in 20 ml. of NaCl-buffer at pH 6.5. A comparison of the hemolytic activity of these preparations is made by measuring the time required for the production of complete hemolysis, at 37°C., in a system containing 0.5 ml. of the final preparation and 0.1 ml. of a suspension made by suspending the thrice washed red cells of 1 ml. of human blood in 20 ml. of NaCl-buffer at pH 6.5. In making these determinations the volume of the control should be the same as that of the experimental system, and the control should be treated in exactly the same manner as the experimental system, except that it should contain only NaCl-buffer at pH 6.5. The results are expressed as the percentage of the control to which the experimental system is equivalent.
tivities is made by entering these times for complete hemolysis on a graph which shows a typical time-dilution curve at 37°C. for the very hemolytic ether-soluble component derived from preincubated mouse liver, acting in a system containing 0.5 ml. of various dilutions of the lytic component and 0.1 ml. of the same human red cell suspension as is used in the systems containing the lysins from fresh or preincubated blood, plasma, or washed red cells (Fig. 1).

The results of the extraction procedures described above have been combined in Fig. 1, and can be summarized as follows:

1. At least two alcohol-soluble substances, one ether-soluble ("soap-like") and the other insoluble in cold ether ("lysolecithin-like") can be obtained from preincubated human blood, plasma, or serum. The hemolytic activity (or concentration, there being no way of deciding between concentration and activity) of the ether-soluble lysin obtained from blood is greater than that of the ether-insoluble lysin, but the activity of the ether-insoluble lysin obtained from plasma or serum is greater than that of the ether-soluble lysin; i.e., the red cells are involved in the case of the ether-soluble material. Lytic material cannot be obtained from preincubated washed red cells alone, however; plasma or serum is required in addition, probably to supply an enzyme which, in the presence of the cells, produces some of the lysin from them during the preincubation period.

2. Preincubation of the blood or plasma for 24 hours at 37°C. increases the yield of lysin two- or threefold. Some relatively weakly lytic material, both of
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The ether-soluble kind and of the ether-insoluble kind, can be extracted from blood or plasma which has not been preincubated; this points to the lytic substances existing as such in normal blood and plasma, and, by inference, in normal tissue. The increase in the activity of the lysins during preincubation is presumably due to the enzymatic splitting off of lysins from lysin inhibitory complexes, and it is quite possible that this is a process which proceeds, slowly and with the accumulation of relatively small concentrations of lytic materials, in normal tissue and blood.

3. When compared in relation to the same time-dilution curve, the ether-soluble lytic material extracted by alcohol from mouse liver is from 5 to 15 times as active as that extractable from blood or plasma, and 20 to 30 times as active as the lysin-inhibitor complexes obtained from mouse liver by extraction with saline. The ether-insoluble substances extracted from liver with alcohol are about twice as active, or occur in about twice the concentration, as those extracted from blood. Comparisons of this kind make one very conscious of the fact that the time which a lyric material takes to bring about hemolysis gives quite a mistaken impression of the activity or concentration of the material. This is due to the shape of the time-dilution curve. One might think, for example, that a lysin such as the ether-insoluble material extracted from preincubated liver, which takes about 20 minutes to produce lysis, is much more active than the ether-insoluble material extracted from preincubated blood, for the latter does not produce complete hemolysis for at least 3 hours. The difference in activity, or concentration, is only about 2.5 times. Looked at in this way, the lysins which Gross (1947) describes as occurring in saline extracts of tumors, which bring about lysis in 20 to 40 minutes, are only about twice as active as those occurring in saline extracts of mouse liver (hemolysis in 2 to 3 hours).

At the same time, it should be remarked that all the lytic substances referred to in Fig. 1 have what would ordinarily be referred to as considerable hemolytic activity. For example, the ether-soluble lysin obtained from preincubated mouse liver has an activity about the same as that of 50 μl of saponin, since it produces hemolysis in from 2 to 4 minutes.

II

Paper Chromatography and Paper Strip Electrophoresis

It is possible to use paper chromatography to separate the soap-like lysins from the lysolecinin-like lysins contained in very small amounts of preincubated mouse liver homogenates or in preincubated mouse blood. It is also possible to use paper strip electrophoresis to separate the protein components of small amounts of preincubated mouse liver, and to ascertain which of them are associated with lytic material. When applied to the study of the components of preincubated mouse liver and preincubated serum, these methods do not
yield much more information than might be obtained in other ways; they are of sufficient interest in themselves, however, to be worth describing, and there are indications already that they will be useful in detecting differences between the lysins and the lysin-inhibitor complexes present in different normal tissues, in tumors, and so on.

1. Paper Chromatography: General Considerations.—If one edge of a piece of dry filter paper (Whatman No. 1, cut into 4 inch squares) is dipped into a suspension of washed red cells in saline, the cells are drawn up evenly as the saline rises by capillarity, although they lag behind the more rapidly advancing saline. Some of the cells hemolyze if, during their upward movement, they pass across a region of the paper which contains a lysin in sufficient concentration; the liberated Hb then diffuses rapidly upwards and tends to accumulate at the very edge of the advancing saline as a thin reddish line. The position of this line marks the position of the region of the paper in which the lysin was contained (Fig. 2 a). If the concentration of lysin is not sufficiently great to produce this phenomenon, a streak of rapidly diffusing Hb may be seen streaming upwards from the area containing the lysin; this streak can be rendered more conspicuous by spraying the paper with a developing agent (a freshly prepared mixture of equal parts of saturated benzidine in 95 per cent alcohol and 5 per cent H₂O₂, with a few drops of glacial acetic acid added).³

The suspension which gives the best results is one made by suspending the thrice washed red cells of 10 ml. of human blood in 50 ml. of saline. About 5 ml. of the suspension is put in a small Petri dish, which is warmed to a temperature of about 37°C. The filter paper containing the lysin is rolled into a cylinder (see below) and is placed upright in the dish containing the suspension. It is kept there for 3 minutes, during which time the cells of the suspension rise through a distance of about 2 cm. The rolled paper is transferred to a second Petri dish containing about 5 ml. of saline, and is kept there until the saline has nearly reached the top of the paper. If the fine reddish line described above appears, the position of the region of the paper which contained the lysin is sufficiently well determined; if it does not, the paper is dried and sprayed with the developing agent.

The color produced by the developing agent fades quickly, and so photography is necessary if permanent records are required.⁴

³ Application of the benzidine-H₂O₂ reagent to the paper as a whole is apt to result in an untidy chromatogram, because the blue color develops in all parts of the paper to which even traces of Hb diffuse. It is neater to shield the paper with a cellophane shield which has a 5 mm. strip cut out of it in such a way as to leave exposed only the part of the paper at which the Hb derived from the hemolyzed red cells is expected to appear (Fig. 2 b). An area of dark blue color marks the position of the lysin, which is to be found somewhere on a line drawn directly downward from the blue area.

⁴ Chromatograms and paper strips are very conveniently photographed with a Land
2. Separation of Soap-Like and Lysolecithin-Like Lysins.—A 10 mm.³ spot of the supernatant fluid of preincubated liver homogenate is placed near the corner D of a paper. The paper is formed into a cylinder with the sides AD and BC bound together, but not touching, with scotch tape, and the side CD is placed in 95 per cent ethyl alcohol as the solvent for the first dimension. After drying, the paper is formed into a cylinder with the sides AB and CD stapled together (since scotch tape is loosened by ether), and the side AD is placed in cold ether as the solvent for the second dimension. When the ether polaroid camera. This camera produces permanent positives in black and white within a minute after the chromatogram is photographed, and so permits one to rephotograph the chromatogram before it fades if the first photograph turns out to be unsatisfactory.

Fig. 2a

Fig. 2b

Fig. 2. Detection of hemolysis in paper chromatograms. (a) The red line of Hb opposite a region containing the alcohol- and ether-soluble component of the supernatant fluid of preincubated mouse liver homogenate. (b) The position of the ether-soluble and ether-insoluble components of the same supernatant fluid marked by the two dark areas on a strip developed with benzidine-H₂O₂.
has ascended about three-quarters of the way up the paper, the paper is dried and reformed into a cylinder with the sides \(AD\) and \(BC\) bound together. The side \(AB\) is placed in the red cell suspension described above, and this is replaced by saline after about 3 minutes. If the position of the lytic components is not marked by red lines in the advancing saline boundary, development is completed with the benzidine-H\(_2\)O\(_2\) reagent. The first hemolytic component is alcohol-soluble but ether-insoluble, \(i.e\.), lysolécithin-like, and is found near the corner \(A\); the second, which is ether-soluble or soap-like, is found about halfway along the side \(AB\). These components give rise to two streams of Hb parallel to the side \(AD\) of the paper (Fig. 2b).

By substituting acetone for ether as the solvent in the second dimension, it can also be shown, although with greater difficulty, that a first lytic component is acetone-insoluble while a second lytic component is acetone-soluble.

3. Failure to Separate the Ether-Soluble Lysins from Liver and from Blood.—These lysins are extracted from preincubated mouse liver and from preincubated mouse blood as already described. The lysin obtained from liver, \(c_l\), is much more active than that obtained from blood, \(b_l\), a hemolytic titration showing, for example, that \(c_l = 5b_l\). One volume (10 mm.) of the lysin \(c_l\) plus 5 volumes of saline is placed as a drop near the corner \(D\) of a paper, 5 volumes of the lysin \(b_l\) plus one volume of saline is placed near the corner \(D\) of another paper, and 6 volumes of a mixture of 1 part of \(c_l\) and 5 parts of \(b_l\) are placed near the corner \(D\) of a third paper.

When the drops have dried, the papers are formed into cylinders with the sides \(AD\) and \(BC\) bound together with scotch tape. The sides \(CD\) are placed in 95 per cent ethyl alcohol as the solvent for the first dimension. After drying, the papers are rolled into cylinders with the sides \(AB\) and \(CD\) bound together, and the sides \(AD\) are placed in the red cell suspension at 37°C. When the cells have ascended about 3 cm., the papers are transferred to saline, the ascent of which completes the development in the second dimension.

In the case of all three papers, a lytic substance which diffuses to the same extent in the second dimension is found about three-quarters of the distance between the drop and the corner \(A\). The possible situation in which the mixture of \(c_l\) and \(b_l\) would give two lytic components found in two regions has not been observed; \(i.e., c_l\) has not been separated from \(b_l\) by paper chromatography.

4. Association of Lysins with Protein Components of Liver.—Chromatography can be used in the following way to show that alcohol-soluble lytic substances are sufficiently associated with proteins to diffuse side by side with them.

Two paper chromatograms of the supernatant fluid of preincubated mouse liver homogenate, of about 3 times the usual concentration, are prepared simultaneously, the 8 inch papers being held in a frame similar to that described by Datta, Dent, and Harris (1950). The proteins are marked by the addition of 0.3 per cent hematin (Franklin and Quastel, 1950), and the volume of the
drops at the corners $D$ is 20 mm. The solvent for the first dimension in the direction $DA$ (arrow 1 in Fig. 3 b) is 3 per cent sucrose. After drying the papers, diffusion in the second dimension (the direction $AB$, arrow 2 in Fig. 3 b) is brought about with water as the solvent. After drying, the positions of the protein components are made visible by spraying with benzidine-$H_2O_2$. Fig. 3 a shows the chromatogram which results, several constituents separating near the corner $B$.

The second paper is now marked with pencil to indicate the position of the protein components found on the first paper, an unnecessary strip of paper above the position of the protein components being cut off. It is then developed in the direction $BC$ with alcohol as the solvent (arrow 3 in Fig. 3 b). After drying and cutting off a strip along the side $CD$, the edge $CD$ is placed in a red cell suspension, and the position of the lytic components is found by observing the position of the thin line of Hb at the advancing boundary (arrow 4 in Fig. 3 b). Fig. 3 b shows the result obtained, and demonstrates that alcohol-soluble lytic material has accompanied the protein material from the drop, first to the corner $A$, and then to the corner $B$, from which it has been extracted with alcohol and carried to the part of the edge $CD$ opposite the position of the protein components. A second smaller accumulation of lyasin is also found on the edge $CD$ but near $D$; this can be accounted for either by supposing that there is more than one alcohol-soluble lyasin (as indeed there seems to be, see section 5 below), or that some of a single material is left associated with the protein (represented by the dark mass near the corner $A$ of Fig. 3 a) which did not have time to diffuse from $A$ to $B$ during diffusion in the second dimension.

5. Paper Strip Electrophoresis.—If two paper strips are mounted side by side in the apparatus described by Durrum (1950), the components of the supernatant fluid of a preincubated mouse liver homogenate which have electrophoretic mobilities can be separated on both strips simultaneously. Both strips are dried, and the positions of the protein components on one strip are found by developing with a fixing and dying solution in the usual way. The second strip is cut into small rectangles which correspond in position to the regions occupied by the protein components on the first strip. Each of the paper rectangles is then extracted with alcohol, and the presence or absence of hemolytic material is ascertained by the addition of washed red cells. In this way it is possible to determine the distribution of lytic materials along the second strip, and to relate this to the distribution of protein components on the first strip. The separation can be carried out at various pH, etc., by using several individual paper strip electrophoresis assemblies.

A fairly good separation of the protein components is obtained by using a homogenate containing about 2 gm. of mouse liver in 5 ml. of NaCl-buffer at pH 6.5. The homogenate is preincubated for 18 hours at 37°C., a clear super-
FIG. 3. (a) Protein components of the paper chromatogram of the supernatant fluid of preincubated mouse liver homogenate. (b) The red line of Hb at the arrow marked 4. This is evidence of the presence of lytic material on the paper below the red line. This lytic material was carried to this position by developing the paper in the direction marked 3, with alcohol as the solvent and is associated with the protein components which occupy the regions outlined in pencil (cf. (a) of this figure). On the original, an indication of the presence of a second accumulation of lytic material can be seen further to the left.
natant fluid is obtained by centrifuging, and 20 mm. of this fluid is applied at the apex of each pair of papers. Mixtures of phosphate buffers (m/20 Na₂HPO₄ and m/20 NaH₂PO₄) are placed in the vessels of the Durrum apparatus. By using 4 assemblies, each with 2 paper strips, separation of components can be carried out at pH 5.6, 6.6, 7.6, and 8.3, but the clearest separations have so far been obtained at pH 5.6 and 8.3. The application of 145 to

![Graph](https://via.placeholder.com/150)

Fig. 4. Paper strip electrophoresis of the supernatant fluid of a preincubated mouse liver homogenate. Abscissae, distance from the apex along the strip in centimeters in the direction of the positive and negative pole respectively. Ordinate, times for complete hemolysis of red cells by the material extracted from these areas of the strips. Upper diagram, at pH 5.6; lower diagram, at pH 8.3. Solid line, proteins; dotted line, lysins.

180 volts for 20 hours results in the fastest of the protein components of preincubated mouse liver moving about 30 mm. from the apex of the strip. The components are not very sharply defined, but there seem to be at least two. At pH 5.6, both components are positively charged; at pH 8.3, the larger component is positively charged while the smaller is negatively charged.

The extraction of the lytic substances from regions of the parallel and undeveloped strip is carried out by marking off both strips into numbered lengths.

5 One of the disadvantages of the strip electrophoresis assembly is that the tendency of the components of the drop to diffuse along the strip is aided by gravity, since the apex is uppermost. This may result in considerable movements of protein and of other components, even when no current is applied, and these movements may mask, or simulate, electrophoretic movements of small velocity.
of about 5 mm., cutting up the undeveloped strip into a series of consecutive numbered rectangles, and placing each in a small test tube to which is added 0.5 ml. of ethyl alcohol. After 2 hours at 37°C., the paper strips are removed, and the tubes are kept at 45°C. until all the alcohol has evaporated. To each tube is added 0.2 ml. of saline and 0.05 ml. of a standard suspension of washed human red cells in NaCl-buffer at pH 6.5; the time required for complete hemolysis at 37°C. is then observed in the usual way.

The results show that there are at least two lytic components, one with an isoelectric point below 5.6 and the other with an isoelectric point above 8.3. They separate very clearly either at pH 5.6 or at pH 8.3, the lysin which is either the more active or present in the higher concentration moving towards the cathode. The unexpected feature of the situation (see Fig. 4), however, is that the regions of the strips from which most lytic material can be extracted do not correspond to the regions which contain most protein. On the contrary, the lysins seem to be found nearly at the extremes of the protein electrophoresis pattern. Similarly, on paper strips to which no current is applied, the lysins are found, not principally at the apex or in regions which the proteins reach by diffusion, but to one side or the other of the apex and just beyond the positions occupied by the protein components.

III

Additional Observations

1. Observations on the Nature of the Lysins.—In the cold, the ether-soluble substances extracted from mouse liver and from serum separate as a white flocculent material from the buffered saline in which they are suspended. The density of this material is usually less than that of the saline, in which it floats. Considerable foaming occurs on shaking. Microscopic examination at 25°C. shows that it is composed of sudanophilic droplets which cohere into larger masses and give the very obvious schlieren seen in saline suspension. All attempts to prepare crystals of the material have failed. These physical properties are compatible with the ether-soluble material being an as yet unspecified mixture of fatty acids and soaps.

An inhibitory effect of CaCl₂ (0.1 M) can be demonstrated if the suspension of ether-soluble material, as finally prepared (see above) and the CaCl₂ are warmed together to 45°C. for a few minutes. At this temperature, the lytic material forms oily droplets and becomes better dispersed than in the cold. This increases the activity of the lytic material itself, the activity measured at 37°C. being greater after the heating than before, and also favors a reaction with Ca, so that on cooling the lytic effect of the Ca-lysin mixture is very much less than when no Ca is added. These observations are compatible with the formation of a relatively non-lytic Ca soap.

2. Inhibition by Serum Components.—The evidence points to the highly
lytic material, both ether-soluble (soap-like) and ether-insoluble (lysolecithin-like), existing in combination with inhibitors as complexes of relatively low hemolytic activity. At least some of these inhibitors are present in serum and plasma, which inhibit hemolysis by both the soap-like and the lysolecithin-like lysins. The inhibitory effects of some of the serum components, measured by methods which have already been described (Ponder, 1945), are shown in Table I. The lecithin (distearyl lecithin) and cholesterol sols were made as described by Lee and Tsai (1942). The protein fractions were kindly sent to me by Dr. E. J. Cohn. Each hemolytic system contained 0.1 ml. of a standard suspension of human red cells in NaCl-buffer (phosphate) at pH 6.5. The appearance of zone phenomena, especially in systems containing the ether-soluble lysins, makes the measurement of the inhibitory effects quite difficult.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Quantity of inhibitor in system</th>
<th>$R$ values</th>
<th>Ether-soluble (soap-like)</th>
<th>Ether-insoluble (lysolecithin-like)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin (distearyl)</td>
<td>50</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>50</td>
<td>1.2</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Fraction I</td>
<td>400</td>
<td>1.5</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Fraction II + III</td>
<td>400</td>
<td>3.0</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Fraction IV + V</td>
<td>400</td>
<td>3.0</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Fraction VI</td>
<td>400</td>
<td>1.5</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

Quantity of lysin = 0.5 ml. of final preparation (see above), diluted 1 in 16 for ether-soluble lysin and 1 in 4 for ether-insoluble lysin.

The total inhibitory effect of serum is approximately what would be expected from the partial concentrations of the protein fractions and their individual inhibitory effects at this pH. Among the protein fractions, the lipoprotein-containing fractions II + III and IV + V are more inhibitory than the others, both for the soap-like lysins and for the lysolecithin-like lysins. Lecithin and cholesterol, on a microgram basis, are more inhibitory than any protein fraction, and cholesterol is considerably more inhibitory for the lysolecithin-like lysins than for the soap-like lysins.

3. Fractionation of Serum and Tissues.—It can be shown, by precipitation of serum or of mouse liver homogenate with (NH$_4$)$_2$SO$_4$, and subsequent extraction of the precipitate and of the supernatant fluid with alcohol and ether, that the soap-like lysins are associated with the globulin fraction, and if human or horse serum is precipitated by Macheboeuf's procedure (Macheboeuf and Rebeyrotte, 1949), the soap-like lysins can be extracted from the crenapses fraction.
Attempts to extract the soap-like lysins from fractions of human serum, kindly provided by Dr. E. J. Cohn, have so far been unsuccessful. Nor has it been possible to take a fraction such as the cenapses fraction, which is not lytic in itself, and to liberate lysin from it by the addition of enzymes or even of small quantities of liver homogenate. Although the soap-like lysins can be extracted, they are apparently bound to an inhibitory part of the complex by bonds which can be broken only under very special conditions; e.g., those prevailing during preincubation of the unfractionated tissue. What these conditions are is quite obscure.

SUMMARY

1. A process of extraction and assay, which combines the features of several existing methods, is described for the lytic materials which can be obtained from blood, plasma, serum, and tissues. At least two alcohol-soluble substances, one ether-soluble ("soap-like") and the other insoluble in ether in the cold ("lysolecithin-like"), can be obtained from preincubated blood, plasma, or serum. The hemolytic activity (or concentration) of the soap-like lysin obtained from blood is greater than that of the lysolecithin-like substance, but for plasma and serum the reverse is true, i.e. the red cells are involved in the production of the soap-like lysin, and probably supply some of it when acted upon by enzymes contained in plasma and serum. Preincubation of the blood or plasma increases the yield of lysin two- or threefold, and small quantities of both soap-like and lysolecithin-like lysins can be obtained from unpreincubated blood or plasma.

2. The soap-like lysins obtained from preincubated mouse liver are some 5 to 15 times as active as, or occur in some 5 to 15 times the concentration of, those obtained from blood or plasma. The lysolecithin-like lysins of preincubated liver are about twice as active as, or occur in about twice as great concentration of, those obtained from blood. Because of the shape of the time-dilution curve for these lysins, the relations between their activities, or concentrations, are often quite different from those which one would anticipate if one were to consider only the times required for the production of hemolysis.

3. Paper chromatography can be used to separate the soap-like and the lysolecithin-like lysins obtainable from small quantities of preincubated mouse liver homogenates or preincubated mouse blood. The presence of lysins is detected by their effect on the red cells of a suspension as it wets the paper. Various technical procedures for separating lytic components and for demonstrating that they move on the paper along with protein components are described.

4. Paper strip electrophoresis can be used to show that the supernatant fluid of a preincubated mouse liver homogenate contains at least two protein
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components and at least two lytic components, not very closely associated
in their electrophoretic behavior.

5. Observations on the physical nature of the alcohol- and ether-soluble
lysin point to its having a soap-like character. Its activity, as well as that of
the lysolecithin-like lysin, is inhibited by cholesterol, by lecithin, and by various
fractions of serum. Some of these effects have been studied quantitatively. The
most inhibitory of the protein fractions are those which contain lipoproteins;
i.e., II + III and IV + V.

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