THE DETERMINATION OF THE COLLOIDAL OSMOTIC PRESSURE IN BLOOD SERUM AND SIMILAR FLUIDS

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The importance of the colloidal osmotic pressure in biological fluids is becoming increasingly recognized, but difficulties in the older methods as yet prevent their general use in inquiries for which needed information can only be obtained from colloidal osmotic pressure data.

The membrane bag arrangement used by Starling (1896) is capable of yielding admirable results (see, e.g., Adair, 1925; Adair and Robinson, 1930), but it requires so much time and material that its use has been limited. The methods in greatest use are those of Schade and Claussen (1924), Govaerts (1923), also Verney (1926), and Krogh and Nakazawa (1927) (see also Wells, 1933; Hill, 1932–33). These all require special apparatus which is not easy to construct and requires considerable skill to use.

In addition to technical difficulties, there is a further point of criticism of the methods in current use in physiological and medical laboratories in that they are based on the assumption that it is desirable to obtain a colloid osmotic pressure reading of the colloid fluid equilibrated against its own ultrafiltrate. The justification of this is based on the assumption that the effective colloid osmotic pressure in the organism is most closely estimated in this way. However, there is no valid proof that this is the case; in this laboratory we have found evidence to indicate that, in man, neither are the capillaries wholly impermeable to blood colloids nor are the colloids alone effective as osmotic agents determining the fluid balance across the capillary membrane. In other words,

1 For brief descriptions of a number of methods, see Meyer (1932).
Colloidal Osmotic Pressure in Serum

the characteristics of artificial membranes are sufficiently different from the membrane of the capillary wall to destroy the principal argument for the ultrafiltrate procedure.

There is a positive argument against the use of the ultrafiltrate procedure in that, when blood serum is used, the variability of the crystalloid composition of the blood serum may alter the relative magnitude of the Donnan effect. If one's desire is to estimate the physical state of the proteins, with regard to apparent molecular sizes, it is obviously desirable to create constant conditions of ionic strength and pH. This was one of our chief concerns in the development of the present method.

Method

The colloid solution (e.g., blood serum) is diluted with an equal volume of a buffer solution of constant ionic strength which is also used as external solution. The wet osmometer cup is rinsed out several times with the diluted colloid solution and then filled to a point where the stopper bearing the manometer tube (see Fig. 1) can be inserted without trapping more than a small bubble of air. The stopper is partly inserted with the upper end of the manometer tube closed with a rubber tube and clamp, and then the end of the tube is opened and the stopper forced in until the fluid stands in the capillary manometer tube higher than the expected colloid osmotic pressure. The outside of the osmometer cup is then carefully washed with the buffer solution to remove all traces of colloid which may have spilled over, and then the osmometer is immersed in the outer (buffer) fluid as shown in Fig. 1.

The equilibration is carried out in an ice box or refrigerator room in which a temperature constant to ±2° is maintained at some level between 0–5°. No manipulations are required, equilibrium being attained spontaneously, owing to the small size of the osmometers and by virtue of the initial approximation of the crystalloid concentration and pH in the inner and outer fluids, before any disturbing bacterial destruction of the proteins intervenes. Sterile technique is, therefore, unnecessary. It is desirable to test the outer fluid for protein at the end of the determination, thereby assuring the acceptability of the results.

The osmometers are read after 3, 4, and 5 days, and the gross colloid osmotic pressure is calculated in mm. of H₂O as colloid osmotic pressure = 2(h - c), where h is the vertical distance in
mm. between $A$ (Fig. 1) and the level of fluid in the capillary manometer tube, and $c$ is the capillarity of the manometer tube, also in mm. of $H_2O$.

The osmometers are extremely simple, consisting of a glass ring to the bottom of which is affixed a collodion membrane ($M$, Fig. 1) and a capillary tube carrying linear graduations in mm. The simplest, and also the best, arrangement for the manometer tube is to have two marks a fixed distance apart ($A$ and $B$, Fig. 1) and a paper mm. scale held in place and protected by an outer glass tube, as shown in Fig. 1.

![Fig. 1. The osmometer mounted as in use. $A$ and $B$ are fixed reference lines on the capillary, the level of the outer fluid being adjusted exactly to $A$. $M$ is the collodion membrane.](image)

The bore of the capillary tube should be about 1 mm. or a trifle less, and its capillarity determined with a diluted colloid solution similar to the fluid to be tested. The capillary tubes should always be scrupulously clean and dry before use.

The membranes are made with a procedure somewhat similar to that used by Adair and Keys (1934). A glass tube of about 3 cm. diameter is mounted to rotate slowly in the horizontal plane; then pour on three coats of collodion, proceeding smoothly from one side to the other, over a length of about 10 cm. The first coat is dried for $1\frac{1}{2}$ minutes after pouring at about $30^\circ$, the second dried for 2 minutes at $30^\circ$, and the third for 5 minutes at $30^\circ$ (an electric heater lamp is excellent for this). Several minutes after the last
drying, the membrane is cut and peeled off as a flat sheet, still quite soft. With the sizes mentioned here, four membranes can be prepared and accordingly the original soft sheet may be cut in quarters.

The soft membrane is laid over the end of one of the glass osmometer rings and molded over the end with the fingers, this molding being repeated several times until the membrane sticks firmly. The excess membrane is then trimmed off and a drop of fresh collodion run around the ring as a final seal. The total drying time in the air following the pouring of the third coat should be between 21 and 24 minutes, and after that the last seal is dried in the air for 1 or 2 hours with the membrane itself just dipping in water.

The collodion solution we use is prepared from 3 parts of du Pont collodion, 1 part of anhydrous ether, and 1 part of ethyl alcohol. It is allowed to stand for several days before use and thereafter keeps indefinitely. If necol collodion is used, the proper proportions are 1.5 to 2 parts of necol to 1 part of ether and 1 part of alcohol. Care should be taken not to get the membrane too thick; a thin mixture is generally better than a thick one.

These membranes should be tested, but, if they are properly made, they are always protein-tight. When not in use, they may be stored for months in 1 per cent HCl in an ice box. Before use they are rinsed in several changes of distilled water for a day or so. These membranes may be used repeatedly, provided they are never allowed to dry once they are prepared.

The suspending and outer buffer fluid may be made up to any desired pH and ionic strength. We have preferred to work with phosphate systems and have used the data of Green (1933) in preparing the solutions. Below are given the compositions, ionic strengths, and pH values for two useful solutions, the first being designed particularly for colloid osmotic pressure studies of cell content solutions, the second being perhaps somewhat better for serum protein studies.

<table>
<thead>
<tr>
<th>Total ionic strength</th>
<th>pH</th>
<th>KH₂PO₄ mole per l.</th>
<th>KOH mole per l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.135</td>
<td>7.2</td>
<td>0.056</td>
<td>0.0395</td>
</tr>
<tr>
<td>0.150</td>
<td>7.4</td>
<td>0.058</td>
<td>0.0460</td>
</tr>
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</table>
The glass osmometer rings we use have an internal diameter of 1.8 cm. and are usually filled to a depth of about 0.4 cm., giving a membrane surface of about 2.5 sq. cm. per cc. of fluid. Determinations in duplicate can be made with about 1.5 cc. of serum.

EXPERIMENTAL

In Table I are given results from a series of osmometers set up with identical serum. These results are more or less typical of the course of equilibration and the final agreement of duplicates.

Typical comparisons of hydrogen ion concentrations of internal and external solutions of human serum are given below, together with colloid osmotic pressure values and pH values by the glass electrode. The effect of pH on the observed osmotic pressure is qualitatively similar to that reported by Marrack and Hewitt (1927).

In forty determinations of colloid osmotic pressure of human blood serum in fifteen resting subjects we have found values for mm. of colloid osmotic pressure per gm. of protein concentration ranging from 39 to 45 with an average of 42.0. This is somewhat lower than is found with the methods of Govaerts (1923), Schade and Claussen (1924), and Krogh and Nakazawa (1927), although

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Table I

<table>
<thead>
<tr>
<th>Serum</th>
<th>Osmometer No.</th>
<th>Initial</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>72 hrs.</th>
<th>96 hrs.</th>
<th>120 hrs.</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>150</td>
<td>220</td>
<td>260</td>
<td>287</td>
<td>291</td>
<td>290</td>
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<td></td>
<td>2</td>
<td>170</td>
<td>240</td>
<td>270</td>
<td>279</td>
<td>286</td>
<td>289</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>400</td>
<td>280</td>
<td>276</td>
<td>284</td>
<td>284</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>350</td>
<td>322</td>
<td>288</td>
<td>296</td>
<td>294</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Colloid osmotic pressure</th>
<th>Internal pH</th>
<th>External pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm. H2O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>285</td>
<td>7.26</td>
<td>7.22</td>
</tr>
<tr>
<td>295</td>
<td>7.39</td>
<td>7.36</td>
</tr>
</tbody>
</table>

* The subjects were all normal young men.
some of this difference disappears when the values are all compared at 37° by multiplication of the colloid osmotic pressure value obtained by the factor 310/(absolute temperature).

It should be remembered that the present method does not measure exactly the same thing as the other methods discussed here, but some comparison of results with the present method and with the second method of Krogh and Nakazawa may be useful. Such comparisons are made in Table II.

The present method invariably gives lower results than the Krogh and Nakazawa method. This may be due partly to the fact that, as has been generally observed (see Meyer, 1932, p. 38), with increasing protein concentration the colloid osmotic pressure increases slightly more than might be expected. The tendency in this direction shown in Table II is general. It is also probable, however, that some of the difference is due to differences in pH and in salt concentrations leading to different Donnan effects.

**SUMMARY**

A true equilibrium method for measurement of the colloidal osmotic pressure in blood serum and similar fluids is presented. Duplicate determinations can be made easily with 2.0 cc. of colloid solution and, with care, a single determination can be made on 0.5 cc. Some results with the method are presented and comparisons are made with other methods.
BIBLIOGRAPHY

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