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Osmotic Pressure of Gum Acacia Solutions.

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Dodds and Haines[1] observed that the colloid osmotic pressure of sterilized 6% acacia solutions was much reduced by added salt, so that in the presence of 0.9% salt the pressure was about one-half that of the blood colloids of mammals. The addition of salt to acacia solutions was, therefore, considered to defeat the object sought in the intravenous administration of the gum,* and the problem becomes of moment in consideration of the use of acacia solutions as a plasma substitute both in physiology and in clinical medicine.

The successful use of acacia in total plasmapheresis experiments on dogs (Stanbury, Warweg and Amberson[2]), and some measurements of colloid osmotic pressure of acacia solutions used in perfusion experiments on frogs (Saslow[3]) suggested that the question be reinvestigated.

Method and Materials. The method used was that employed by Dodds and Haines,[1] namely, the second method of Krogh and Nakazawa.[4] The method is adequate to the purpose though admittedly far from ideal. The precision attained was of the same order as that reported by Wies and Peters,[5] namely, a standard deviation of ± 8 mm H_2O in a series of 20 determinations on one sample of 6% acacia made up in 0.9% NaCl (the colloid osmotic pressure of the sample at 20°C was 260 mm H_2O).

The membrane used was cellophane, plain, No. 600. During the course of the experiments the material was kept at 21°C and 30 to 40% relative humidity in the Precision Instrument room of the Marine Biological Laboratory. Before use, pieces of cellophane were soaked in the appropriate crystalloid solution for several hours.

1 Dodds, E. C., and Haines, R. T. M., *Biochem. J.*, 1934, **28**, 499.

* On the basis of their measurements, the object of intravenous administration of the gum would have been defeated by the salts present in blood, whether or not salt were added to the acacia before injection.

2 Stanbury, J., Warweg, E., and Amberson, W. R., *Am. J. Physiol.*, 1936, **117**, 230.

3 Saslow, G., *Am. J. Physiol.*, 1938, **124**, 360.

4 Krogh, A., and Nakazawa, F., *Biochem. Z.*, 1937, **188**, 241.

5 Wies, C. H., and Peters, J. P., *J. Clin. Invest.*, 1937, **16**, 93.

The cellophane was tested for acacia-tightness with an ultrafilter operated at 60 kg per square centimeter for 3 hours. To the ultrafiltrate was added a few drops of 3% lead subacetate (Ritsema[6]); a well marked precipitate is formed with acacia in concentrations down to 0.05%. The test is unreliable in dilutions of acacia greater than this. Test of numerous samples of the ultrafiltrate was negative, *i. e.*, less than 0.05% acacia is forced through the cellophane under the conditions described. If we take the osmotic pressure of acacia solutions as roughly linear with concentration (actually the pressure falls off at the low concentrations), the maximum error which could have occurred with supposed leakage of acacia of the order of 0.05% is 2 mm H_2O.

The acacia used for most of the measurements was part of a special lot, containing no added NaCl. This lot was prepared for us by the Eli Lilly Company from one large batch of gum and was used in perfusion experiments described recently (Saslow[3]). The mode of preparation of the acacia was as follows: The acacia used is described as "highest quality pearls, Grade No. 1"; the pearls were hand-selected for color, those with a dark tint being discarded. 1750 g of the selected acacia pearls were dissolved in 5 liters of water containing 25 g sodium glycerophosphate; the water was warmed to approximately 75°C to effect solution. The solution was autoclaved for 4 hours at 120°C, and then allowed to stand over night. A precipitate formed which was filtered off; the filtrate was adjusted to pH 6.4 *to 6.7 with* NaOH. The solution was heated on the water bath at 60°C for 15 minutes and then kept at 4° over night. The slight precipitate which formed was filtered off and the solution was diluted with sufficient freshly distilled water so that each 100 cc contained 27 to 30 g of solids. The diluted solution was filtered until clear and filled into ampoules. These were sealed and sterilized by boiling one-half hour on each of 3 successive days. The composition of this lot of acacia in "30% solution" was as follows: total solids, 26.8%; total ash, 1.0%; total base, 20.8 cc N/1 acid per 100 cc; Ca, 0.147%; K, 0.198%; Na, (by difference) 0.186%; Mg, 0.0025%; PO_4, less than 0.0004%; unidentified H_2S metals as Cu, 0.025%. According to the experience of the Eli Lilly Company, the range of composition of different samples of 30% acacia similarly prepared is: Ca, 0.126 to 0.136%; Mg, 0.0015 to 0.0038%; pH, 5.0 to 5.2; PO_4, none; heavy metals, none by the tests in U.S.P. XI.

For purposes of comparison, the colloid osmotic pressure of sev-

[6] Ritsema, C., *Pharm. Weekblad.*, 1934, **72**, 105.

eral other lots of acacia solution was measured. These were prepared with added NaCl, by the Lilly Research Laboratories.

Measurements were made at temperatures of 21 to 25°C. In any one run, the variation in temperature was of the order of 1°. Final readings were taken from 20 to 24 hours after the osmometers had been set up. Trial showed that the colloid osmotic pressure became steady at about 16 hours and remained so for at least 70 hours. All values tabulated (Tables I and II) are the average of 6 to 10 individual measurements, and have been reduced to 20°.

Results. The colloid osmotic pressure of the special lot of acacia (referred to hereafter as Lot 1) in NaCl solutions of various concentrations is shown in Table I.

TABLE I.
Colloid Osmotic Pressure of 6% Acacia (Lot 1) in NaCl Solutions.

NaCl %	pH outside fluid	pH inside fluid	Colloid osmotic pressure mm H_2O	Dodds and Haines[1] Colloid osmotic pressure mm H_2O	Dodds and Haines[1] pH inside fluid
0.00	5.58	5.60	773	841	4.5
0.05	5.63	5.51	555	531	—
0.10	5.50	5.47	462	350	—
0.15	5.60	5.43	415	—	
0.20	5.51	5.41	365	232	—
0.28			—	196	4.4
0.30	5.58	5.43	304	—	
0.31			—	193	—
0.46			—	170	4.3
0.50	5.49	5.38	285	—	
0.70	5.52	5.35	285	—	
0.90	5.50	5.32	260	128	4.2
1.00	5.49	5.31	238	—	
2.00			—	118	—

The colloid osmotic pressure of acacia Lot 1 was measured also in buffered Ringer-Locke's of the following composition: NaCl, 0.9%; KCl, 0.042%; $CaCl_2$, 0.024%; P(Na_2HPO_4, NaH_2PO_4) 10 mg %; pH 7.20; $\mu = 0.176$; $\Delta = 0.699°$. The value obtained is compared in Table II with the colloid osmotic pressure of different lots of

TABLE II.
Colloid Osmotic Pressure of Different Lots of Acacia.

No. of lot	Date sealed into ampoules	Date measured	Solution measured	Colloid O.P. mm H_2O
1	9/28/37	8/6/38	6% acacia in Ringer-Locke's	243
1	”	6/23/38	6% ” , 0.9% NaCl	260
2	10/7/37	8/3/38	6% ” , 0.9% ”	246
3	1/11/38	8/17/38	6% ” , 0.9% ”	253
4	7/22/38	9/2/38	6% ” , 0.9% ”	250

acacia prepared with added NaCl (the measurements were made on solutions diluted to 6% acacia, 0.9% NaCl).

The figures in Table II should be compared with some determinations of Wies and Peters,[5] who measured the colloid osmotic pressure of a sample of Lilly-prepared acacia with added NaCl and diluted to 6% acacia, 0.9% NaCl; the pressure at 20° was 252 mm H_2O. A similar value was obtained by Turner (quoted by Wies and Peters[5]) on another sample. Butt and Keys,[7] using a sample of acacia the origin and preparation of which are not stated, obtained the value 262 mm H_2O for 6% acacia, 0.9% NaCl.

The colloid osmotic pressure of 6% acacia in 0.9% NaCl is approximately the same as the pressure of human serum proteins, as reported by Wies and Peters.[5] In their series of observations, the colloid osmotic pressure of 69 sera the serum protein concentrations of which lay between 6 and 8% covered the range 205 to 404 mm H_2O, with the average value at 276 mm H_2O.

The method used for the measurements described appears to give the same result as the much more laborious method (due to Adair) used by Butt and Keys[7] so far as the acacia available in the United States is concerned. Further, acacia prepared according to the procedure described seems to be a uniform and stable product, having in 6% solution in media approximating the salt content of blood about the same colloid osmotic pressure as serum itself.

That added salt lowers the colloid osmotic pressure of acacia solutions has been observed by several workers. A particularly complete study of the subject has been made by Oakley.[8] His experiments show that the osmotic pressure of acacia solutions depends greatly, as is to be expected of a polyvalent colloidal electrolyte, upon the qualitative and quantitative salt content of the medium, the pressure being greater the smaller the added salt content of the solution. As added salt is increased, the ionic pressure difference between colloid solution and dialysate decreases continuously. The precise form of the relation between the osmotic pressure and the added salt concentration will depend not only upon the amount and nature of the salt added but also upon the initial composition of the acacia solution itself. Should this have been so prepared as to have a relatively high total ash content, the osmotic pressure will fall more sharply and to lower levels upon the addition of given amounts of salt than if the original solution had a lower total ash content. An acacia solution

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[7] Butt, H. F., and Keys, A. J., *Physical Chem.*, 1938, **42**, 21.

[8] Oakley, H. B., *Trans. Faraday Soc.*, 1934, **31**, 136; 1936, **32**, 1360; 1937, **33**, 372.

of lower initial pH will also show a lower osmotic pressure than one of higher pH (Oakley[8]). Since the composition of the acacia solutions used by Dodds and Haines[1] is not given in sufficient detail, it is impossible to say more than that very likely the explanation of the difference between their values for the colloid osmotic pressure of 6% acacia in 0.9% NaCl and the values of the various investigators cited here lies in differences in inorganic composition of the initial acacia solutions. Presumably the same explanation applies to the marked differences among the values reported for the colloid osmotic pressure of 6% acacia solutions by various investigators (quoted by Amberson[9] and Butt and Keys[7])—values ranging from 120 to 3740 mm H_2O; some of the differences arose, however, because measurements were made on solutions now of one salt content, now of another. Recent measurements made in the United States on 6% acacia in 0.9% NaCl or similar solutions seem to be in satisfactory agreement.

Summary. 1. The colloid osmotic pressure of several samples of 6% acacia in 0.9% NaCl has been determined to be 246 to 260 mm H_2O at 20° C. 2. This value is approximately the same as the average colloid osmotic pressure of human sera ranging in protein concentration from 6 to 8%, namely, 276 mm H_2O. 3. Acacia solution processed by the procedure of the Lilly Research Laboratories appears to be a stable and uniform product.

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Evidence of Another Factor in the B Complex for Rats.

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B_1 (Thiamin), flavin, nicotinic acid, B_6, and the filtrate factor are generally accepted as distinct components of the B complex for rats. Recent work on other species of animals by Morgulis, Wilder, and Eppstein[1] and by Jukes and Babcock[2] indicates that antimuscular dystrophy and antiparalytic factors exist, while Stockstad[3] reports evidence of a distinct growth factor U for the chick.

9 Amberson, W. R., *Biol. Rev.*, 1937, **12**, 48.
1 Morgulis, S., Wilder, V. M., and Eppstein, S. H., *J. Nutr.*, 1938, **16**, 219.
2 Jukes, T. H., and Babcock, S. H., *J. Biol. Chem.*, 1938, **125**, 169.
3 Stockstad, E. L. R., and Manning, P. D. V., *J. Biol. Chem.*, 1938, **125**, 687.