the test animals were found to give positive tuberculin tests after intervals of 4 and 8 weeks. Recovery of acid-fast organisms was made from the liver, spleen and lymph nodes.

3. The cultivation of the organisms on Sweaney's egg-glycerol medium.

Three separate series of tests were made on each type of membrane. Two guinea pigs were used for the controls and 2 for each test. Each animal was previously found to be tuberculosis-free as determined by the tuberculin test. An even emulsion of the organism was obtained by thoroughly grinding the growth as washed from the solid media in physiological saline.

Our results show that the ultrafiltrates did not contain infectious particles capable of producing: (1) a positive tuberculin reaction; (2) anatomical evidences of the infection in experimental animals or growth of tubercle bacilli on appropriate media for their cultivation. It may be concluded, therefore, that the cultures tested did not develop "filterable forms" smaller than 40 μμ in diameter.

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Colloidal Calcium Phosphate of Blood and Calcium Partition in Serum.

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It has now been thoroughly shown that under certain conditions the inorganic phosphate of the serum becomes incompletely diffusible.[1] A logical explanation of this phenomenon is that the nondiffusible phosphate which is formed is combined with some of the serum calcium in a compound which is in a colloidal state. As yet, no direct evidence is available as to the exact composition of this colloidal calcium phosphate.

On the basis of the view which has recently received strong support from the work of McLean and Hastings,[2] namely, that there is an equilibrium between the ionized and the protein bound calcium in

[1] See Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, in press, for literature.

[2] McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 529; *J. Biol. Chem.*, 1934, **107**, 337.

serum which is governed by the mass law, it appears possible to develop a relationship which will quantitatively account for the partition of the serum calcium and one which will also yield a plausible value for the composition of the colloidal calcium phosphate. In the following treatment it is assumed that the diffusible calcium is virtually equivalent to the ionized calcium of the serum. This opinion also is supported by the work of McLean and Hastings.

For the present purpose, the mass law relationship may be conveniently written in the form,

$$\frac{P}{CaP} = \frac{1}{A} + \frac{B}{Ca_D^{++}} \qquad (1)$$

where P represents the protein, CaP the calcium protein complex, and Ca_D^{++} the diffusible calcium. A and B are constants whose significance is given below. The equation as given has the advantage that if the observed data obey the mass law, then a straight line is obtained on plotting the values of $\frac{P}{CaP}$ against $\frac{1}{Ca_D^{++}}$. The value of the constant A is given by the reciprocal of the point of intersection of the straight line on the P/CaP axis. It has the significance of being the maximum amount of calcium which can be bound by each unit weight of protein. The slope of the straight line gives the value of the constant B. This term consists of the equilibrium constant divided by A.

The application of this theory as applied to drawn beef blood serum, which has been augmented with varying amounts of calcium, is illustrated by the data given in Table I. The results of 4 experimental series on blood taken from different animals are given in the table.

The most satisfactory results were obtained when the colloidal calcium phosphate was assigned a composition of $Ca_3(PO_4)_2$. The amount of non-diffusible calcium present in this form in any sample was calculated from the non-diffusible inorganic phosphate content of the serum. The value so obtained, subtracted from the total non-diffusible calcium, yields the protein-bound calcium.

The values of the protein-bound calcium determined in this manner were plotted against the diffusible calcium values according to the method which has been indicated. The plot yielded the numerical equation,

$$\frac{P}{CaP} = 0.51 + \frac{3.5}{Ca_D^{++}} \qquad (2)$$

where the protein content is expressed in grams and the calcium

TABLE I.
Calcium and Inorganic Phosphate Partition in Beef Serum Augmented with Calcium.

Series	Protein %	Calcium: Serum	Calcium: Diffusible mg. %	Calcium: Non-diffusible	Inorganic Phosphate: Serum	Inorganic Phosphate: Diffusible mg. % of P	Inorganic Phosphate: Non-diffusible	$Ca_3(PO_4)_2$ calcium	Protein Bound Calcium: Experimental	Protein Bound Calcium: Calculated
A-1	7.35	11.2	4.8	6.4	5.55	5.55	0	0	6.4	5.9
A-2	7.3	15.6	6.6	9.0	5.55	4.5	1.05	2.0	7.0	7.0
A-3	7.3	20.0	8.8	11.2	5.5	3.8	1.7	3.3	7.9	8.0
A-4	7.2	29.9	12.9	17.0	5.5	1.4	4.1	7.9	9.1	9.2
B-1	8.6	11.3	4.0	7.3	6.6	6.1	0.5	0.9	6.4	6.2
B-2	8.5	19.4	8.6	10.8	6.55	2.75	3.8	7.3		
B-3	8.45	29.3	10.9	18.4	6.5	1.8	4.7	9.1	9.3	10.1
B-4	8.35	38.3	15.3	23.0	6.4	0	6.4	12.4	10.6	11.2
B-5	8.30	47.1	21.5	25.6	6.4	0	6.4	12.4	13.2	12.4
C-1	6.75	11.7	6.0	5.7	7.5	7.5	0	0	5.7	6.2
C-2	6.65	20.4	7.3	13.1	7.4	4.0	3.4	6.6	6.5	6.7
C-3	6.60	29.2	11.2	18.0	7.3	1.8	5.5	10.6	7.4	8.0
C-4	6.55	37.7	17.2	20.5	7.3	1.3	6.0	11.5	9.0	9.2
C-5	6.50	46.4	24.0	22.5	7.2	0.7	6.5	12.5	10.0	9.9
D-1	6.9	9.3	4.0	5.3	7.7	7.7	0	0	5.3	5.0
D-2	6.9	14.1	5.5	8.6	7.7	6.25	1.45	2.8	5.8	6.0
D-3	6.85	17.9	6.6	11.3	7.65	5.0	2.65	5.1	6.2	6.6
D-4	6.8	22.3	7.8	14.5	7.6	3.85	3.75	7.2	7.3	7.1
D-5	6.8	26.6	10.7	15.9	7.55	3.25	4.3	8.3	7.6	8.1

values in milligrams per 100 cc. of serum. From the A value in equation (2) there is obtained the information that 1.96 mg. of calcium should be the maximum amount capable of being bound per

gram of beef serum protein. This result would yield a value of 14 mg. % for the protein-bound non-diffusible calcium in a serum with a protein concentration of 7.2%. This figure harmonizes with a previous deduction of Greenberg and Gunther[3] that with increasing calcium ion concentration the protein-bound calcium increases only by a moderate amount. From the value of B in equation (2) the equilibrium constant of the mass law relationship is found to have the value of $10^{-2.75}$ when expressed in units of mols per liter. This is of the same order of magnitude as the value of the equilibrium constant found by McLean and Hastings for human blood serum.

The degree to which the experimental data fit the equation given is indicated by the results in the last column of Table I, under the heading, "Protein Bound Calcium, Calculated." From a comparison of the figures calculated according to equation (2), which are given in this column, with the experimentally obtained values, it is seen that the agreement is satisfactory. The results which are presented appear to favor the viewpoint that the colloidal calcium phosphate found in blood serum has the composition of the tertiary salt and that the equilibrium between the protein-bound and the ionized calcium conforms to the mass law.

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Kidney Glomerulus of Hypertension Produced Experimentally by Pituitary Excess.

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That the thickened basement membrane of glomerular capillaries in hypertension is primarily due to the hypertension itself has not been definitely shown and the suggested relationship of the pituitary to hypertension has been based upon insufficient clinical evidence. The present experiments have produced in the amphibian a condition paralleling hypertension in the mammal with vasoconstriction, slowed heart rate, thickened ventricular wall and edema (Blount[1]). This has been accomplished by the transplantation of additional pituitary anlagen in the embryo (Blount[2]) which develop and give

[3] Greenberg, D. M., and Gunther, L., *J. Biol. Chem.*, 1930, **85**, 491.

[1] Blount, R. F., *Anat. Rec.*, 1933, **55**, Suppl. 7.

[2] Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113.