

## PROTEIN FILMS ON COLLODION MEMBRANES.\*

BY DAVID I. HITCHCOCK.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, April 21, 1925.)

### I.

#### INTRODUCTION.

It was observed by Loeb<sup>1</sup> that collodion bags which had been filled with gelatin solutions and then washed many times with water behaved differently from untreated collodion bags in experiments on the rate of diffusion of electrolytes and water through the collodion. This behavior seemed to indicate the existence of a film of protein adhering to the collodion. Loeb obtained similar results by treating collodion bags with solutions of other proteins, such as casein, egg albumin, blood albumin, edestin, and oxyhemoglobin. In the latter case the existence of the film was made evident by the persistence of a red coloration on the membrane. The film can be rendered visible in the case of other proteins by an experiment suggested by J. H. Northrop, which consists in placing a piece of the protein-treated and washed membrane in acetone; the collodion rapidly dissolves, leaving the insoluble protein as a bulky opaque residue. The present work was undertaken in order to study some of the variables on which this film formation depends.

### II.

#### EXPERIMENTAL METHOD.

The proteins used were gelatin and egg albumin, purified<sup>2</sup> by the methods of Loeb and Sørensen, respectively. The membranes were flat disks of collodion, prepared from a solution made by diluting

\*Presented before the Leather and Gelatin Division of the American Chemical Society at Baltimore, April 8, 1925, and published here by permission of the Editor of the *Journal of the American Chemical Society*.

<sup>1</sup> Loeb, J., *J. Gen. Physiol.*, 1919-20, ii, 255, 577.

<sup>2</sup> Loeb, J., *Proteins and the theory of colloidal behavior*, New York, 1st edition, 1922; 2nd edition, 1924.

Merck's collodion (U. S. P. IX) with an equal volume of a mixture of 75 parts by volume of ether with 25 parts of 95 per cent alcohol. This gave a solution containing about 2 gm. of dry collodion in 100 cc. The membranes first used were prepared on a circular glass plate 10.1 cm. in diameter, levelled by being floated in a Petri dish full of mercury. 10 cc. of the collodion solution were allowed to flow from a pipette on to the glass plate, which was shielded by a paper cylinder<sup>3</sup> (enclosing also a small open vessel containing ether) for the few seconds required for the collodion to spread over the surface of the glass. The shield was then removed, the time noted, and the solvents were allowed to evaporate from the collodion for 15 minutes at room temperature (about 20°C.) At the end of this time the plate and membrane were put in water, and the membranes were kept in water until the following day, when they were used.

Each membrane was blotted between filter papers, rolled loosely, and put into a Pyrex test-tube with 25 cc. of a protein solution. The test-tubes were tightly closed by rubber stoppers and rotated end over end for about 16 hours in a water thermostat at 37°C.  $\pm 0.02^\circ$ . The amount of protein adhering to each membrane was determined by removing the membranes, washing each by stirring a few times in 300 cc. of water at 37°C., drying for 1 hour in an oven at 100°C., and weighing the dry membrane with its adherent protein. At the same time two or three untreated membranes were similarly dried to obtain the dry weight of the collodion alone. The weights of membranes from the same lot always agreed to 1 or 2 mg., being within 5 mg. of 0.200 gm. Membranes dried 2, 4, and 21 hours differed in weight by less than 1 mg. from those dried 1 hour. This method for determining the weights of protein was checked to about 2 per cent by dry weight determinations of gelatin solutions before and after shaking with the membrane, the gelatin samples being dried for 24 hours at 100°C.

### III.

#### EFFECT OF PROTEIN CONCENTRATION.

Experiments were carried out by the above method with solutions of isoelectric gelatin and egg albumin of different concentrations.

<sup>3</sup> Nelson, J. M., and Morgan, D. P., Jr., *J. Biol. Chem.*, 1923-24, lviii, 305.

When the egg albumin solutions were shaken with collodion membranes at 37°C., it was found that part of the protein was denatured and precipitated out. Accordingly the egg albumin solutions were not shaken, but simply left in contact with the membranes at 37°C. Each membrane was cut in halves so that it might be completely submerged in the solution. Some typical results are given in Fig. 1. The curves appear to have the general shape found in many types of adsorption experiments. It was found, however, that the results

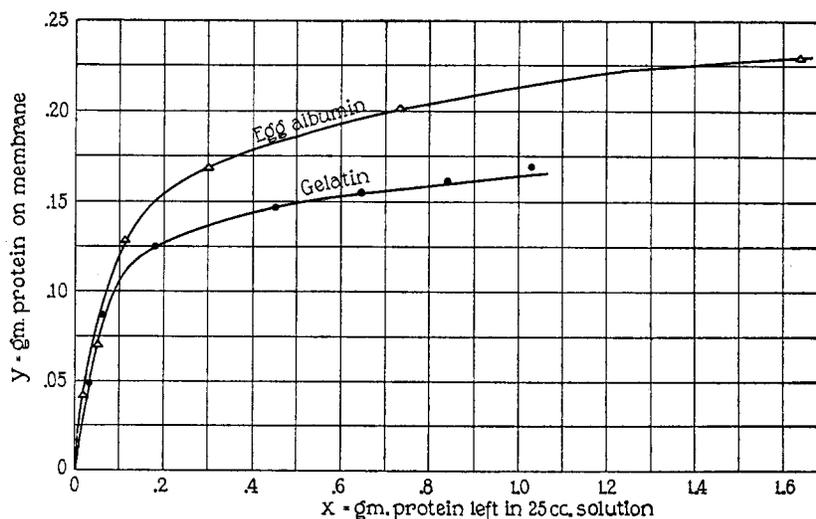


FIG. 1. Effect of protein concentration on the adsorption of gelatin and egg albumin by collodion membranes.

could not be fitted by the hyperbolic equation of Freundlich,<sup>4</sup> which is often referred to as the adsorption isotherm. His equation has the form  $y = kx^{\frac{1}{n}}$ , where  $y$  is the amount of substance adsorbed by a fixed amount of adsorbent,  $x$  is the concentration of the solution at equilibrium, and  $k$  and  $n$  are constants to be determined from the experimental values of  $x$  and  $y$ . The fact that this equation does not apply to the experiments of Fig. 1 is shown by Fig. 2, where the logarithms of the amounts adsorbed are plotted against the logarithms

<sup>4</sup> Freundlich, H., *Kapillarchemie*, Leipsic, 3rd edition, 1923, 151, 232.

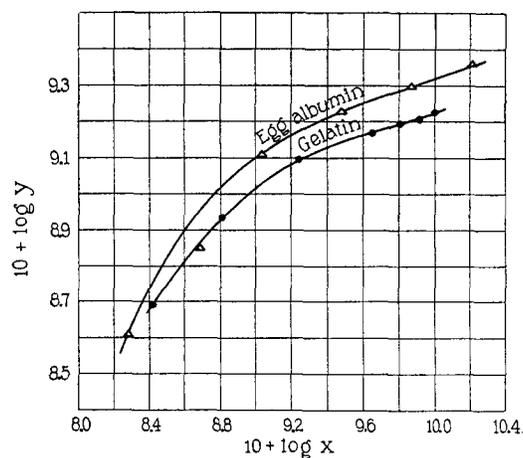


FIG. 2. Test of the application of Freundlich's equation,  $y = kx^{\frac{1}{n}}$ , to the data shown in Fig. 1. Since the curves are not straight lines, the equation does not apply to these experiments.

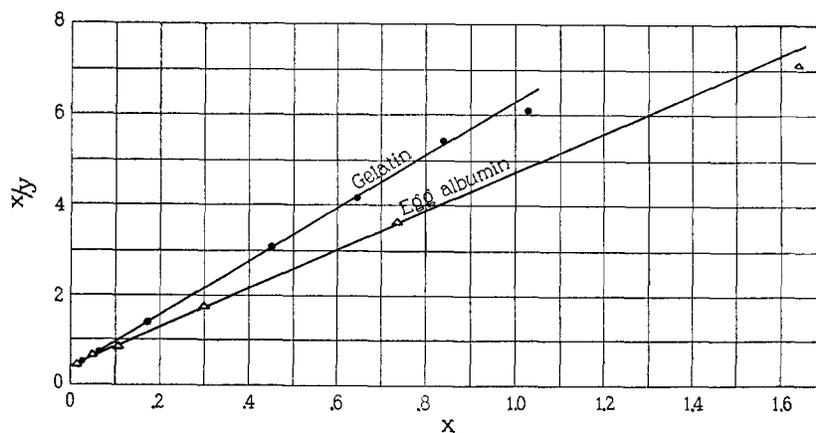


FIG. 3. Test of the application of Langmuir's equation,  $\frac{x}{y} = \frac{1}{ab} + \frac{x}{b}$ , to the data shown in Fig. 1. Since the curves are straight lines, the equation applies to these experiments.

of the final concentrations. If the equation applied, the points of each experiment should fall on a straight line.

Fig. 3 represents a test of the applicability to the same data of an

equation of hyperbolic type derived by Langmuir<sup>5</sup> for the adsorption of a gas on a plane surface. This equation has the form

$$y = \frac{a b x}{1 + a x} \quad \text{or} \quad \frac{x}{y} = \frac{1}{a b} + \frac{x}{b}$$

where  $x$  and  $y$  have the same meaning as before and  $a$  and  $b$  are constants. In Fig. 3 the values of  $\frac{x}{y}$  are plotted against those of  $x$ , and if the equation applies, the data should give straight lines. The figure shows that this is very nearly the case. The value of  $\frac{1}{a b}$  is given by the intercept on the axis of ordinates, while the value of  $\frac{1}{b}$  is the slope of the line. This equation of Langmuir predicts that in higher concentrations the amount is adsorbed,  $y$ , should reach a constant limiting value,  $b$ , while Freundlich's equation does not. In the experiments to be described below, in which smaller membranes were used, such limiting values were definitely obtained.<sup>6</sup>

#### IV.

##### EFFECT OF HYDROGEN ION CONCENTRATION.

The effect of hydrogen ion concentration on the removal of protein from solution was determined with solutions containing varying amounts of HCl or NaOH together with 0.5 gm. of gelatin or egg albumin in 25 cc. The weight of the protein film was obtained directly as outlined above, and the pH of each solution was determined with the hydrogen electrode at 25°C. The results are given in Fig. 4. In each case the amount of protein adhering to the membrane was greater at the isoelectric point than at any other pH.

The effect of different acids on the amounts of gelatin adhering to the collodion is shown in Fig. 5. The points obtained with CH<sub>3</sub>COOH and H<sub>3</sub>PO<sub>4</sub> are quite close to the curve for HCl, while the curve for H<sub>2</sub>SO<sub>4</sub> is considerably higher.

<sup>5</sup> Langmuir, I., *J. Am. Chem. Soc.*, 1918, xl, 1368, 1384.

<sup>6</sup> It may be noted that an equation of the same form as this adsorption equation of Langmuir would result from the law of mass action as applied to a reversible combination between two substances, one at a fixed total concentration, the other, variable.

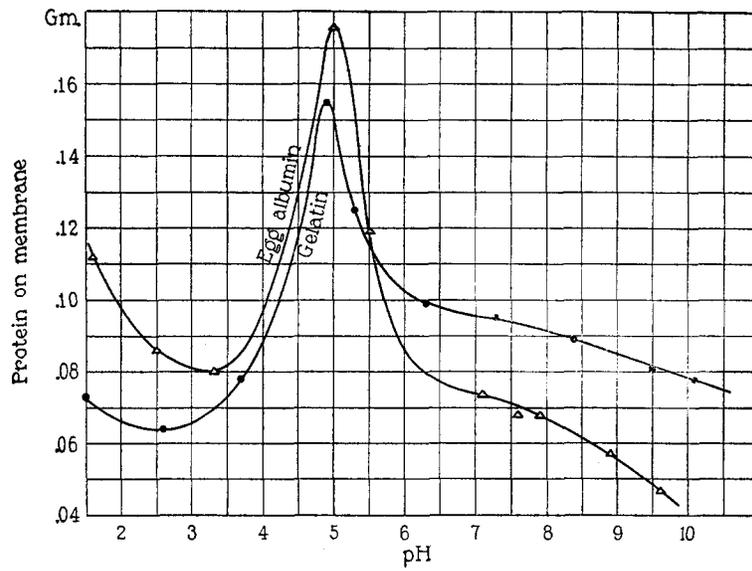


FIG. 4. Effect of pH on the adsorption of gelatin and egg albumin from 2 per cent solutions by collodion membranes.

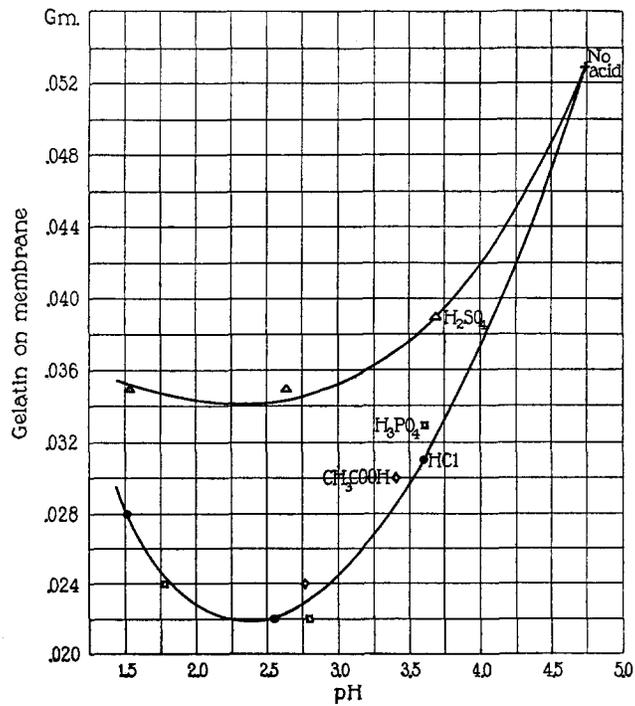


FIG. 5. Effect of different acids on the adsorption of gelatin from 2 per cent solutions by collodion membranes.

Fig. 6 shows the effect of salt concentration on the adsorption of gelatin from 2 per cent solutions containing 0.02 M HCl, pH about 2.5. While the values obtained in low concentrations of salt are erratic, it is evident from the figure that each salt tended to increase the amount adsorbed up to the value obtained at the isoelectric point with no salt,  $\text{Na}_2\text{SO}_4$  having more effect than NaCl. In this experiment the weight of the combined ash of the thirteen protein-coated membranes was found to be only 0.001 gm., which shows that the increased weight obtained was not due to adherent salt.

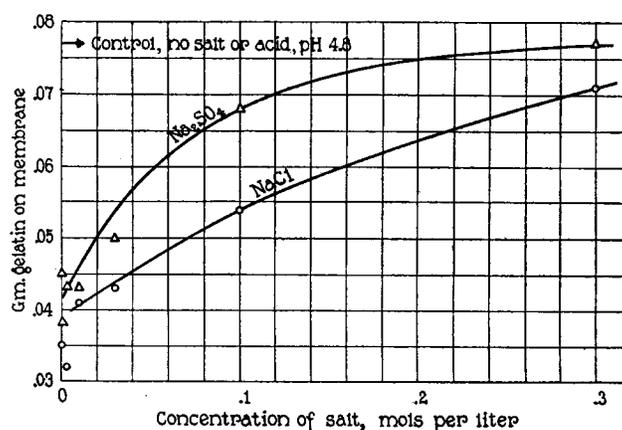


FIG. 6. Effect of salts on the adsorption by collodion membranes of gelatin from 2 per cent solutions in 0.02 M HCl (pH 2.5).

The curves in Figs. 4, 5, and 6 show that the amount of gelatin adhering to the collodion membranes was affected by the same variables which Loeb<sup>2</sup> found to affect the viscosity and other colloidal properties of gelatin solutions, but in an opposite direction. Fig. 7 gives a comparison of the effect of pH on the amount of adherent gelatin and on the fluidity of 2 per cent gelatin solutions which had been kept overnight at 37°C. The fluidity values, in c. g. s. units, were calculated from relative viscosity determinations made with Ostwald viscometers, by means of Bingham's<sup>7</sup> data for the absolute viscosity of water. Except for the high fluidities at low pH, which

<sup>7</sup> Bingham, E. C., Fluidity and plasticity, New York and London, 1922.

are probably due to hydrolysis of the gelatin, the two curves show a marked parallelism.<sup>8</sup>

The explanation for these results is still uncertain. The following suggestion may be tentatively made. If Loeb's explanation for the viscosity of gelatin solutions at 20° or 24° applies to solutions at 37°, then the effect of pH is to change the size of submicroscopic particles by affecting the degree of swelling conditioned by the Donnan equilib-

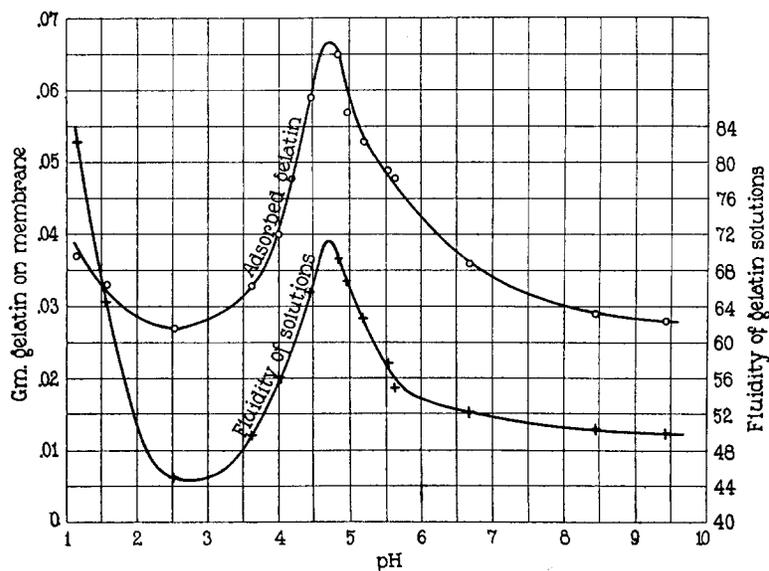


FIG. 7. Effect of pH on the fluidity of gelatin solutions and on the amount of gelatin adsorbed by collodion membranes.

rium. Accordingly when the viscosity is at a minimum the particles are smallest, and hence more particles can get in contact with the surface of the membrane and adhere. In the case of egg albumin the explanation is probably different, for with this protein Loeb found no marked effect of pH on viscosity. More experiments will be required before any explanation for these pH curves can be advanced with certainty.

<sup>8</sup> A coincidence of minimum adsorption with maximum viscosity in alkaline solutions has been reported (Abderhalden, E., and Fodor, A., *Kolloid-Z.*, 1920, xxvii, 49) in the case of the adsorption by charcoal of casein and a yeast protein. In acid solutions of the latter the addition of salt increased the adsorption.

## V.

## EFFECT OF PERMEABILITY OF MEMBRANES.

In experiments with membranes made on different days by the method described above, the results were not reproducible. This irregularity was found to be due to variations in the permeability of the membranes caused by variations in the temperature and humidity of the laboratory when the membranes were prepared. It was found that as the time of drying the membranes before immersion in water was increased, their capacity to adsorb gelatin decreased. Membranes which had been allowed to become very impermeable, by being dried for 1 hour or more, did not take up any measurable quantity of gelatin.

In order to obtain membranes of uniform permeability, a method of preparation was adopted which had been suggested by Bigelow<sup>9</sup> and improved by Bartell and Carpenter.<sup>10</sup> 109 cc. of the collodion solution described above (2 gm. in 100 cc.) were poured on the surface of mercury in an enamelled iron tray about 27 × 33 cm., all bubbles were scraped over to the edge of the dish with a knife, and the dish was covered with a cardboard case about 32 × 49 × 50 cm. This retarded the evaporation of the solvents so that the time of drying was from 2 to 7 hours at about 20°C. At the end of this time water was poured directly on the collodion and the membrane was cut away from the sides of the tray and kept in water at least 1 day before use. These large membranes were cut into disks with a steel die 3.81 cm. in diameter. In each test-tube 3 disks were shaken with 25 cc. of gelatin solution, so that the apparent surface of the collodion was less than half of that used in the previous experiments. The dry weight of 3 disks varied from 0.073 to 0.089 gm., being determined separately for each membrane.

While the disks of each membrane were being shaken with the gelatin solutions, measurements of thickness and permeability were made with other disks of the same membrane. The thickness was determined by cutting a straight edge with scissors and observing the thickness of the edge with a micrometer microscope. Several measure-

<sup>9</sup> Bigelow, S. L., *J. Am. Chem. Soc.*, 1907, xxix, 1675.

<sup>10</sup> Bartell, F. E., and Carpenter, D. C., *J. Phys. Chem.*, 1923, xxvii, 101, 252.

ments were made with different freshly cut edges, and could be reproduced within 0.008 mm.

The permeability measurements were made at room temperature by forcing water through the membrane in an apparatus similar to that of Bartell and Carpenter.<sup>10</sup> The area of membrane exposed to pressure had a diameter of 2.1 cm., the highest pressure used was about 75 cm. of water, and the rate of flow in a horizontal tube of 0.792 mm. diameter was determined with the aid of a stop-watch and a millimeter scale. The rate of flow was found to be quite closely proportional to the pressure applied, which is in agreement with the ob-

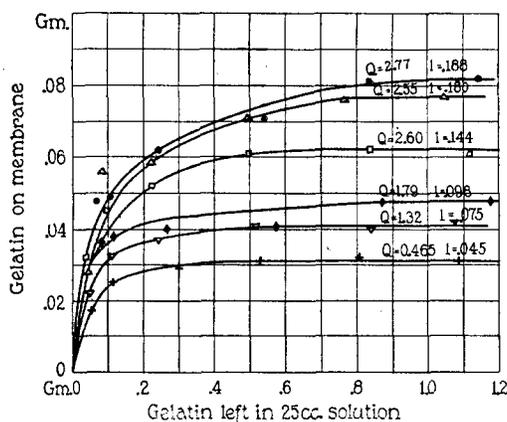


FIG. 8. Adsorption of gelatin by membranes of different permeabilities.

servations of Bigelow,<sup>9</sup> of Bartell and Carpenter,<sup>10</sup> and of Duclaux and Errera.<sup>11</sup>

The results of adsorption experiments with gelatin and membranes of different permeabilities are given in Fig. 8. Each curve is marked with the permeability of the membrane,  $Q$ , expressed in cu. mm.  $\times 10^{-4}$  of water flowing through an area of 1 sq. cm. of membrane per second per mm. of water pressure, and with the thickness in mm.,  $l$ , of the membrane. Each curve indicates a definite maximum amount of adsorbed gelatin, being thus qualitatively in agreement with Langmuir's equation. In general the maximum amount of gelatin

<sup>11</sup> Duclaux, J., and Errera, J., *Rev. gén. colloïdes*, 1924, ii, 130.

adsorbed increased with the permeability and the thickness, the more permeable membranes being thicker.

It seemed of interest to compare the maximum amounts of adsorbed gelatin with the relative surface of the pores in the different membranes, as calculated from the permeability and thickness measurements by means of Poiseuille's law, which is

$$V = \frac{p \pi r^4 t}{8 L \eta}.$$

This law applies to the flow of a liquid through a capillary tube, and in the equation  $V$  = volume of liquid flowing in time  $t$  under pressure  $p$  through a tube of radius  $r$  and length  $L$ ;  $\eta$  represents the coefficient of viscosity of the liquid. If the membrane is considered as a bundle of capillary tubes, the permeability as defined above is equal to  $\frac{nV}{pt}$  where  $n$  is the number of tubes per sq. cm. of membrane area. Hence the permeability,  $Q$ , is given by the equation

$$Q = \frac{n \pi r^4}{8 L \eta}.$$

The length of the capillaries,  $L$ , is not known, but if it be assumed proportional to the thickness of the membrane,  $l$ , then  $Ql$  is proportional to  $r^4$ , or  $r \propto \sqrt[4]{Ql}$ , if the number of pores per sq. cm. of the membranes is taken as constant, which is in accordance with the conclusion of Bartell and Carpenter.<sup>10</sup> Hence the total pore surface of 1 sq. cm. of membrane,  $2 \pi rLn$ , is proportional to  $l r$ , or to  $l \sqrt[4]{Ql}$ . Fig. 9 shows a plot of the maximum amounts of adsorbed gelatin against the relative pore surface, or  $l \sqrt[4]{Ql}$ . The fact that the points lie nearly on a straight line seems to indicate that the maximum gelatin adsorbed is a linear function of the pore surface. This line cannot be extrapolated back to the axis of ordinates, for it was found that membranes of low pore surface adsorbed no gelatin. Hence the curve becomes discontinuous near its left end. Probably the discontinuity would lie at the point where the pores become too small to admit any gelatin at all.

Some measurements were made of the permeabilities of membranes, initially identical, after they had been treated with gelatin solutions of varying concentration. Fig. 10 shows that the square root of the

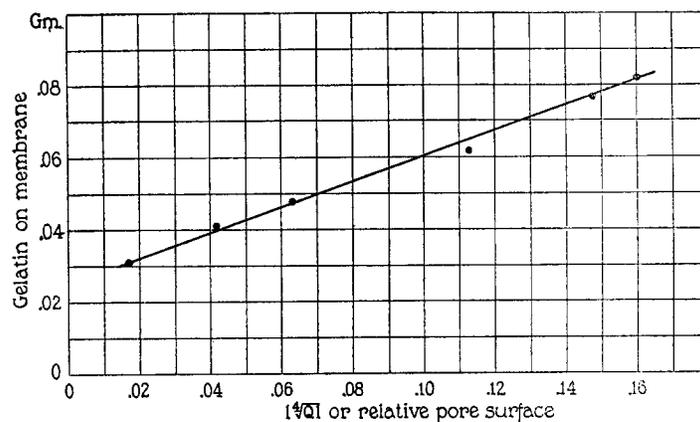


FIG. 9. Adsorption of gelatin as a function of relative pore surface as calculated from Poiseuille's law.

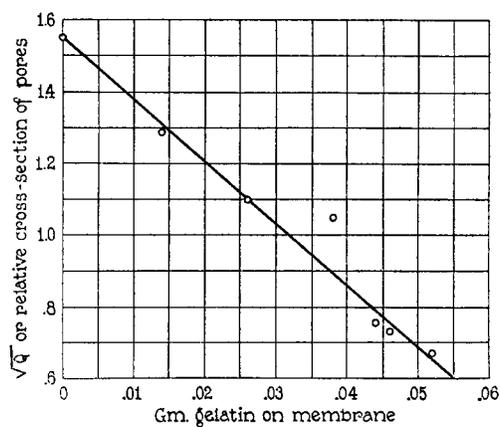


FIG. 10. Relative cross-section of pores of gelatin-coated membranes as a function of the amount of adherent gelatin.

permeability,  $Q$ , obtained as described above, was a linear function of the amount of gelatin on the membrane. Since in this case the thickness of the membrane,  $l$ , was constant,  $\sqrt{Q}$  is proportional to  $r^2$  if it is

assumed that the total number of pores remained the same. Hence the experiment shows that the cross-section of the pores left open after treatment with gelatin is a linear function of the amount of adherent gelatin. Thus Figs. 9 and 10 both confirm the idea that the adsorbed gelatin formed a layer on the surface of the pores. From the first and last points of Fig. 10 it can be calculated that the gelatin decreased the pore cross-section by 57 per cent or the radius by 34 per cent. On extrapolating the data of this experiment by means of Langmuir's equation and Fig. 10, it appears that the maximum amount of gelatin (0.068 gm.) which this membrane could have adsorbed would have decreased the pore cross-section by 76 per cent or the radius by 51 per cent.

#### SUMMARY.

1. Collodion membranes of high permeability were found to adsorb weighable amounts of gelatin and egg albumin from solution at 37°C.
2. The effect of protein concentration could be expressed fairly well by a hyperbolic equation proposed by Langmuir for the adsorption of gases by a plane surface, while the usual parabolic adsorption equation of Freundlich did not fit the results.
3. In comparing this effect with solutions of varying pH, it was found there was a decided maximum of adsorption in solutions of isoelectric protein. The effects of acids and salts on the amount of gelatin adsorbed were like those observed by Loeb on the viscosity of gelatin solutions, but opposite in direction. The effects of pH on the amount of adsorbed gelatin and on the fluidity of the gelatin solutions were nearly parallel.
4. Membranes made impermeable by long drying took up very little or no gelatin from solution.
5. In the case of membranes of varying permeability the maximum amount of adherent gelatin increased with the permeability and thickness of the membranes, and appeared to be, within limits, a linear function of the relative pore surface of the membranes as calculated from Poiseuille's law.
6. The film of gelatin greatly decreased the permeability of the membranes, as measured by the flow of water through them. The relative cross-section of the pore openings, as calculated from the

permeability measurements, was a linear function of the amount of adherent gelatin. These results led to the conclusion that the gelatin formed a film inside the pores.

Many of the experiments described in this paper were carried out by Mr. C. E. Heinrichs. The writer is indebted to Dr. J. H. Northrop for advice in connection with the work.