and its contents was compared with the weight of the same balloon and a bowel loop of similar length to that strangulated. Here the increase in weight is due solely to the blood lost into the wall of the loop, into the lumen, and outward into the balloon. Twelve cats were used in this series. Two of these, in which leakage at the neck of the balloon gave death from peritonitis, were discarded. In one, where the whole small intestine was strangulated, and the balloon contents compared with intestines of similar length in animals of the same weight, the blood loss was estimated at 45% of the blood volume. In 2 animals, strangulation of one-half of the small intestine gave a blood loss of 43% and 52% of the blood volume. Such a blood loss is in itself sufficient to cause death. In 7 smaller loops, each including one-third of the small intestine, the blood loss into the balloon varied from 22% to 35% of the estimated blood volume —a considerable but not a fatal loss. Using the same technique, Holt[2] found an even greater blood loss in the strangulated intestine of the dog.

Conclusions. Blood loss from the general circulation is an unimportant factor in simple occlusion of the intestine, and in closed loop obstruction without strangulation. In venous strangulation in the cat, blood loss is sufficient to cause death if one-half or more of the small intestine be involved. In strangulation even of smaller loops, the blood loss into them, and through them, while not in itself great enough to cause death, is yet sufficient to be a factor of importance. In clinical cases of strangulation, probably some part of the bloodstained transudate is reabsorbed by the peritoneum. No attempt was made in these experiments to estimate or to allow for such reabsorption.

8306 C

Quantitative Spreading of Fibrinogen in Unimolecular Films.

LYMAN FOURT* AND ANNE M. PERLEY. (Introduced by Francis O. Schmitt.)

From the Department of Zoology, Washington University, St. Louis.

Two general methods have been employed to spread proteins on aqueous surfaces in unimolecular films. Gorter and Grendel[1] in-

[2] Holt, R. L., *Brit. J. Surg.*, 1934, **21**, 582.

* University Fellow.

[1] Gorter, E., and Grendel, F., *Proc. Acad. Sci. Amsterdam*, 1926, **29**, 1262.

troduced the method of blowing the protein solution horizontally upon the surface of the water in a Langmuir trough. Hughes and Rideal[2] placed on the water surface solid particles of the protein previously weighed on a microbalance. They could show that the films so obtained, while fluid at the outset, became elastic upon compression (sol-gel transition). Not all proteins, however, are amenable to spreading; examples are fibrinogen[3, 4] and nerve nucleoprotein.[5] These proteins spread very slowly and cannot be induced to form sol, *i. e.*, fluid films. Instead, small amounts float about in elastic patches with equilibrium surface pressures of less than 0.1 dyne/cm., as compared with a pressure of 16 dynes/cm. for gliadin (Hughes and Rideal[2]). The tendency to spread is so slight that because of its greater specific gravity the protein solution usually sinks into the buffer in the trough.

It is highly desirable that methods be worked out for the quantitative spreading of such proteins. In attacking this problem we have worked with fibrinogen because of its ready availability and because methods for its purification are at hand. The fibrinogen was prepared according to the method of Hammarsten as modified by Florkin[6] except that oxalated beef blood was used instead of citrated horse plasma. As recommended by Mellanby[7] all salt solutions were oxalated; by this means the fibrinogen could be reprecipitated 3 times without denaturation. To apply the film we used a 0.2 ml. serological pipette graduated in thousandths. The pipette was sealed to one arm of a stopcock ground for vacuum; the other arm received a small hypodermic needle which was bent so that the protein solution was delivered horizontally with the pipette vertical. Films were spread on McIlvaine buffer, diluted tenfold. All pH determinations were made with the glass electrode.

Confirming the original report of Gorter,[3] fibrinogen films studied under these conditions could not be spread quantitatively. In searching for methods of facilitating the spreading of the protein we tried adding a small amount of alcohol before applying the film. The mixture then spread rapidly, the alcohol quickly evaporating or diffusing into the underlying liquid. Fibrinogen solution containing 10% alcohol showed no precipitate even after 10 hours; with alcohol concentration 20%, precipitation was rapid.

---

[2] Hughes, A. H., and Rideal, E. K., *Proc. Roy. Soc. London*, 1932, **A137**, 62.

[3] Gorter, E., *Am. J. Dis. Child.*, 1934, **47**, 945.

[4] Gorter, E., and v. Ormondt, H., *Biochem. J.*, 1935, **29**, 48.

[5] Fourt, L., Bear, R. S., and Schmitt, F. O., *Am. J. Physiol.*, 1935, **113**, 44.

[6] Florkin, M., *J. Biol. Chem.*, 1930, **87**, 629.

[7] Mellanby, J., *Proc. Roy. Soc. London*, 1935, **B117**, 352.

TABLE I.

Alcohol %	3.6		7.1		9.5	
pH	6.9		2.9		4.9	
	Protein Applied mg.	Area per mg. m^2	Protein Applied mg.	Area per mg. m^2	Protein Applied mg.	Area per mg. m^2
	.0096	.811	.0096	.770	.0258	.631
	.0120	.745	.0144	.749	.0379	.597
	.0168	.741	.0192	.686	.0439	.679
	.0192	.695	.0264	.720	.0540	.657
	.0240	.687	.0336	.653	.0568	.632
	.0278	.674	.0420	.582	.0572	.664
	.0342	.649	—	—	.0602	.608
Average Area		.716		.695		.638
Film Thickness		12.7 Å		13.8 Å		14.6 Å

The table illustrates typical results obtained with such films by extrapolation of the linear portion of the surface pressure—area curves to zero pressure. Protein was determined by micro Kjeldahl, using a factor of 6.25. Thickness of film was calculated by assuming the density of fibrinogen to be 1.1. That the fibrinogen was spread quantitatively is shown by the fact that the area per milligram is essentially independent of the amount of protein spread. This is particularly true with the alcohol concentration of 9.5%; with lower concentrations of alcohol, spreading was slower and maximum spreading was prevented by the formation of an elastic restraining ring of protein film, within which subsequent protein emitted from the pipette was held under compression.

The results as tabulated are comparable to those previously reported by Gorter.[4] He has found for most of the proteins examined a maximal spreading of 1.0 m^2./mg. at the isoelectric point, and a minimal spreading of 0.1 m^2./mg. on either side of the isoelectric point. Our figures are intermediate. The isoelectric point of beef fibrinogen is as yet undetermined, but our preparations showed maximum flocculation in the range of pH 4.2-5.6. According to Hughes and Rideal[2] gelation of gliadin sets in at a thickness of 11-12 Å and an area of about 0.67 m^2./mg. This supports the view that the fibrinogen films reported above were always in the gel state, since the area lies between 0.72 and 0.64 m^2./mg. and the thickness between 12.7 and 14.6 Å.