unusually coarse, a limitation being placed on the fineness of the grouping by the fact that measurements cannot be made with a precision greater than about 0.2μ, the limit of resolution. Thus the volume distribution which can be obtained in hypotonic plasma are not capable of deciding whether it is the increase in cell volume, or the stretching of the cell membrane, which is proportional to the initial cell volume.

In the course of the investigation it occurred to me that the final result might be influenced by the fact that all the small (or large) cells of the same animal do not necessarily contain the same amount of W, nor do they all necessarily have the same value of R. I accordingly computed what would happen if the values of RW were distributed with a coefficient of variation of 20%, and if the values were uncorrelated with cell size. The computations are exceedingly tedious, but the result is the same as that stated above: as the tonicity is reduced, there appears in the volume distribution of the intact cells a small skewness which would be experimentally undetectable.

Conclusion. The experimental frequency distributions for red cell volume obtained with spherical forms in hypotonic plasma in which there are varying degrees of hemolysis are compatible either with the idea that the volume at which the cell hemolyses is proportional to its initial volume, or with the idea that the stretching of the cell membrane at the moment of lysis is proportional to the initial cell volume.

8290 C

Nature and Permeability of Grasshopper Egg Membranes. II. Chemical Composition of Membranes.*

THEODORE LOUIS JAHN. (Introduced by J. H. Bodine.)

From the Zoological Laboratory, State University of Iowa.

The secreted membranes surrounding the diapause egg of the grasshopper, *Melanoplus differentialis,* consist of 4 distinct layers: the exochorion, a thin microscopically homogeneous layer; the endochorion, an underlying granular layer; a thin (about 1.5μ) yellow cuticle; and a thick white fibrous cuticular layer.[1] These can be fur-

* Aided by a grant from the Rockefeller Foundation for work on cellular physiology.

ther subdivided.[2] The chorion is secreted by the mother, and the cuticle is secreted by the serosa cells when the embryo is about 6-11 days old at 25°C. The only published observation on the chemical composition of the membranes is that of Campbell,[3] who obtained a positive chitosan test with the cuticle (both layers combined) but not with the chorion. Since at least 2 of these membranes are semi-permeable[4] their chemical compositions are of considerable importance in interpreting data on permeability of the egg.

The chorion could be separated from the cuticle mechanically, but no method was found for separating the layers of the chorion. When eggs were soaked overnight in concentrated HCl and washed in water for several hours the white fibrous cuticle could be separated from the others in a small percentage of cases. The thin yellow cuticle was freed from the others by boiling in concentrated HCl. The solubilities and some of the chemical reactions of the layers have been investigated, and the results, together with the chemical reactions of chitin (from various sources, loc. cit.) are shown in Table I. Determination of the isoelectric point was made

TABLE I.
Chemical Reactions.

		Grasshopper membranes				
	Reagent	exo-chorion	endo-chorion	yellow cuticle	white cuticle	Chitin
38%	HCl, cold	sl. sol.	sl. sol.	insol.	sol.*	sol.
80%	HNO_3, "	" "	" "	v. sl. sol.	v. sl. sol.	"
93%	H_2SO_4, "	" "	" "	insol.	sol.*	"
38%	HCl, hot	sol.	sol.	"	"	"
80%	HNO_3, "	"	"	sol.	"	"
93%	H_2SO_4, "	"	"	insol.	"	"
70%	KOH, cold	sl. sol.	sl. sol.	"	insol.	insol.
70%	KOH, 160-190° C.	sol.	sol.	ins. 15 min. sol. 90 min.	"	"
	Chitosan	—	—	—	+	+
	Diaphanol-Zn-Cl-I	—	—	—	—	+
	Millon's	+	±	—	—	—
	Liebermann's	±	+	—	—	—
	Xanthoproteic	+		—	—	—
	Isoelectric point	2.0 ca.	3.7 ca.	none	none	3.5

Key:
sol. = dissolved within 10 minutes.
sl. sol. = dissolved within 2 weeks.
v. sl. sol. = visible disintegration in 2 months.
insol. = not dissolved within 2 hours in hot or 2 months in cold solutions.
* = with the exception of a very thin portion which dissolved only slowly.

1 Slifer, E. H., and King, R. L., *J. Morph.*, 1934, **56**, 593.

2 Slifer, E. H., unpublished observations.

3 Campbell, F. L., *Ann. Entomol. Soc. Am.*, 1929, **22**, 401.

4 Jahn, T. L., *J. Cell. and Comp. Physiol.*, 1935, in press.

by the prussian blue method of Loeb.[5] Pieces of chorion and cuticle were left for 24 hours in citrate buffers over the pH range 1-5 to which a few drops of weak $K_4Fe(CN)_6$ had been added. They were then washed 24 hours in buffer solution without cyanide and treated with $FeCl_3$. Exposures of only 1-2 hours in buffer-cyanide solutions as used by Yonge[5] were ineffective, probably because of the low permeability of the exochorion. Discoloration of the chorion produced by the action of $FeCl_3$ did not allow a very definite end point, so that the isoelectric point values are only approximate. Attempts to obtain a color reaction above the isoelectric point with $CuSO_4$ and $K_4Fe(CN)_6$ were unsuccessful, probably because of rather effective masking of color by the normal deep yellow of the chorion. The cuticle, however, was not masked and did not stain by either method; that is, it did not act like an ampholyte in this pH range as did the chitin of decapods in the experiments of Yonge.[5]

It can be seen from the table that the 2 layers of the chorion are probably composed of 2 proteins, the exochorion giving a positive Millon and a doubtful Liebermann reaction and the endochorion a positive Liebermann and a doubtful Millon reaction. These were observed microscopically on the combined layers. The combined layers of the chorion gave a positive xanthoproteic reaction, but low intensity of the color did not permit microscopical determination of the responsible layer. Biuret tests were all negative, probably because of the extreme insolubility of the material. The isoelectric point of the exochorion was about 2.2, while that of the endochorion was about 3.7. The value for the exochorion is one of the lowest isoelectric points known for a protein, and it agrees with the value of 2.2 postulated on the basis of electrical HCl-KCl concentration effects.[4] This agreement, although only approximate, supports the theory that modified diffusion potentials are the source of the observed concentration effects. On the basis of this theory the concentration effect with KCl should be positive above and negative below the isoelectric point of the membrane. This was found in previous experiments.[4]

The chitosan tests of Campbell[3] (confirmed by Slifer, unpublished observation) indicated that at least one of the 2 layers of the cuticle contained chitin, and one of the objects of the present investigation was to determine which of the 2 layers this was. The thin yellow layer is eliminated by its high insolubility. It remained brownish-yellow in boiling HCl and turned black but did not dissolve in con-

[5] Yonge, C. M., *Proc. Roy. Soc. London*, 1932, B**111**, 298.

centrated H_2SO_4, and it was only slowly soluble in concentrated KOH at 160-170°C. These reactions, together with the negative chitosan and diaphanol tests and the failure to stain below pH 3.5 in the prussian blue reaction very definitely distinguished it from chitin. Lack of a high resistance to boiling HNO_3 indicates that it does not have the same chemical structure as the cuticulin of Rhodnius,[6] although it may be closely related. Failure to stain by the prussian blue method below pH 5.5 distinguished it from the cuticle of the decapods.[5]

The white fibrous layer of the cuticle is similar to chitin in regard to solubility except that it dissolves very slowly in cold concentrated HNO_3, a month or longer being required for a visible disintegration to occur. The van Wisselingh-Brunswik-Campbell methods for detecting chitin (KOH at 160-180° for 15 minutes followed by I_2-KI in 1% H_2SO_4 which gives a violet color, and then solution in 75% H_2SO_4 and the subsequent precipitation upon dilution of small spherical crystals, supposedly chitosan sulphate, which have affinity for acid dyes) gave positive results with this layer of the cuticle, thus confirming the observations of Campbell and of Slifer. The diaphanol-zinc-chlor-iodide method of Schulze,[7] however, never gave a positive reaction. Membranes were left in diaphanol as long as 5 weeks and were subsequently placed in zinc-chlor-iodide for one minute or longer (up to 12 hours) before washing in water, but no definite violet color was obtained with either of 2 samples of diaphanol (Grübler) used. Sometimes a salmon-red color was observed as the zinc-chlor-iodide solution was diluted, but this was always faint and never remained even for a short time when the membranes were placed in water. The acetic acid-bisulphite modification of Koch[8] also gave negative results. The nymphal exoskeleton, however, gave a positive Schulze reaction after 24-48 hours in diaphanol, thus showing that the reagents and technique were satisfactory. The composition of the white cuticle, therefore, is probably similar to but not identical with the chitin of the exoskeleton.

The nymphal exoskeleton is impregnated with proteins and gave a positive Millon reaction. The cuticular layers of the egg, however, did not give positive Millon, Liebermann, or xanthoproteic reactions.

The contradictory results obtained with these microchemical

[6] Wigglesworth, V. B., *Quart. J. Microscop. Sci.*, 1933, **76**, 269.

[7] Schulze, P., *Z. Morph. u. Ökol. Tiere*, 1924, **2**, 643.

[8] Koch, C., *Z. Morph. u. Ökol. Tiere*, 1932, **25**, 730.

tests for chitin bring up the question of the specificity of the reactions employed. According to Campbell[3] the chitosan methods are very specific. Koch,[8] however, claims that the method of Schulze is more specific than those recommended by Campbell, and he cites the tracheae of *Apis millifera* and *Musca domestica* which gave negative chitosan but positive diaphanol-zinc-chlor-iodide reactions. The white layer of the cuticle of the grasshopper egg gives the reverse of these results. This indicates that there are probably 3 substances or chemical groupings which may be detected by either one or the other of these tests (or only 2 if they are both present in cases where both reactions are positive).

8291 C

Behavior of the Spindle Fibers in Centrifuged Cells.

BASILE J. LUYET.

From the Department of Biology, St. Louis University.

As I described in a previous paper,[1] by centrifuging the cells in mitosis, in the onion root tip, for one hour with a force equivalent to 30,000 times the force of gravity, I obtained a displacement of the chromosomes in the centrifugal direction, a concentration of the cytoplasm in 2 layers at the lower portion of the cell, and a separation of the cell sap to the upper region. What happens to the mitotic spindle when the cells are treated under the same conditions is the subject of this communication. The method used is, in all its details, the same as that already described.[2]

In the spindles and spindle fibers so treated I observed the following facts: 1. Only the upper cone of the spindle is present (Fig. 1, A-E), the lower one not appearing. 2. The height of the cone often exceeds one-half, and can be as much as three-fourths, of the height of the cell (Fig. 1, B). However, all the intermediate heights between this maximum and the short cones of the prophase stage are present. 3. I never observed fibers broken or badly distorted or folded on themselves, and rarely were they entangled. They are often slightly bent, but in a smooth curve. 4. The fibers stay together as the hair of a moist tapering brush; they are never scattered in different directions. 5. The half spindle bores its way cen-

1 Luyet, B. J., and Ernst, R. A., *Biodyn.*, 1934, **2**, 8.

2 Luyet, B. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1225.