

tion. The initial titer of the antisera ranged from 1 : 24 to 1 : 512, but in some cases these antisera were diluted 1 in 6 or more prior to absorption. Absorptions at first were performed for an hour or more at room temperature, followed by a period of varying length (usually over night) in the refrigerator, later for one half hour at room temperature. Two absorptions were sometimes necessary. Agglutination tests were run in hanging drops or in small vials, readings in every case being made under the microscope at from 15 to 30 minutes after the addition of sperm to the antisera. Agglutinations were classified as 0, \pm , +, ++, +++, or +++++, the latter indicating that all or nearly all the motile sperm were struck together in mats or clumps. Adhesion was usually by tails or middle-pieces, but it was not uncommon to see active sperm pairs accurately aligned and closely adherent throughout their lengths, and in a few cases adhesion was almost exclusively by heads. There was no indication of any effect on motility.

Of 9 antisera tested, 4 have given positive results, 4 have given negative results, and 1 has given doubtful

results. The results with 3 of the 4 positive sera are summarized in Table 1. It will be seen that anti-C57

TABLE 1

Sperm injected	Sperm absorbed with	Sperm tested against			
		F ₁ (C57 \times P)	C57	C	P
C57	C57		0		
Same	C		++++	\pm	
C57	C		++++	+	+++
F ₁ (C57 \times P)	C	+++	++++	\pm	+++
Same	C57	+++	\pm	\pm	++
Control	C		0	0	0

sperm serum absorbed with C sperm clearly differentiates between C57 and C sperm, strongly agglutinating the former, but leaving the latter largely free swimming. Whether one or more antigens is involved is not yet clear. The results with anti-F₁(C57 \times P) sperm serum point to the presence of a second distinguishing antigen or group of antigens in the P sperm.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A CLOSED CELL FOR ELECTRON MICROSCOPY

ONE of the severest disabilities of the electron microscope has consisted in the fact that the specimen is exposed to high vacuum and consequently is completely desiccated and perhaps destroyed before observation. For many purposes it is desirable to study a specimen in its original medium without such desiccation and possible alteration. For this purpose an enclosed chamber is necessary.

A very simple closed chamber for electron microscopy has here been devised. The plastic windows interfere very little with electron examination, and they are liquid and vapor tight and easily withstand a difference of one atmosphere pressure between the inside of the cell and the remainder of the electron microscope. A fuller account is being communicated to *The Journal of Applied Physics*.

The cell¹ is shown in Fig. 1. It consists of two circular discs of platinum one eighth inch in diameter. The large annular groove in the lower disc holds a ring of adhesive wax (Cenco "Tackiwax") and excess liquid from the specimen. When the protruding annular ridge of the cover is pressed down upon this, the wax forms a vacuum-tight seal. Before the two

¹ The cells, together with jigs and supports used with them, are supplied by Mr. J. Grebmeier, Instrument Maker, Menlo Park, Calif. They fit existing RCA microscopes. An alternative mechanical seal avoids wax which melts.

discs are pressed together it is necessary to place a thin collodion film upon each. Ordinary collodion films are worthless, but the following procedure yields strong vapor-tight membranes about 500 Ångstroms thick.

Two drops of a 1 per cent. solution or one drop of a 2 per cent. solution of Baker U.S.P. collodion cotton in purified amyl acetate is allowed to spread on thoroughly cleaned mercury, nine centimeters square. The mercury is previously washed three or four times in a long column of 10 per cent. potassium hydroxide, followed by washings with 1N nitric acid, hot and cold water, and finally drying. Mercury and film are kept as free as possible from dust. The average thickness of films is less than six hundred Ångstroms. A rough indication of the film strength can be obtained by puncturing the film with a sharp point. If the point passes through without encountering any resistance, the film is weak; if it is stopped momentarily with wrinkles radiating out from the point of contact, then the film is relatively strong.

To place the films as a window covering a 0.1 millimeter hole, a centrally bored platinum disc is simply raised upward through the surface of the mercury. Scoring the film beforehand with a sharp needle facilitates its removal.

Contrast in a liquid medium is necessarily far less, although the membranes still permit sharp photographs of dry colloidal gold particles. Likewise, for

ultramicroscopic particles, free Brownian movement in a liquid as fluid as water is far too large and rapid to permit of photographic recording.

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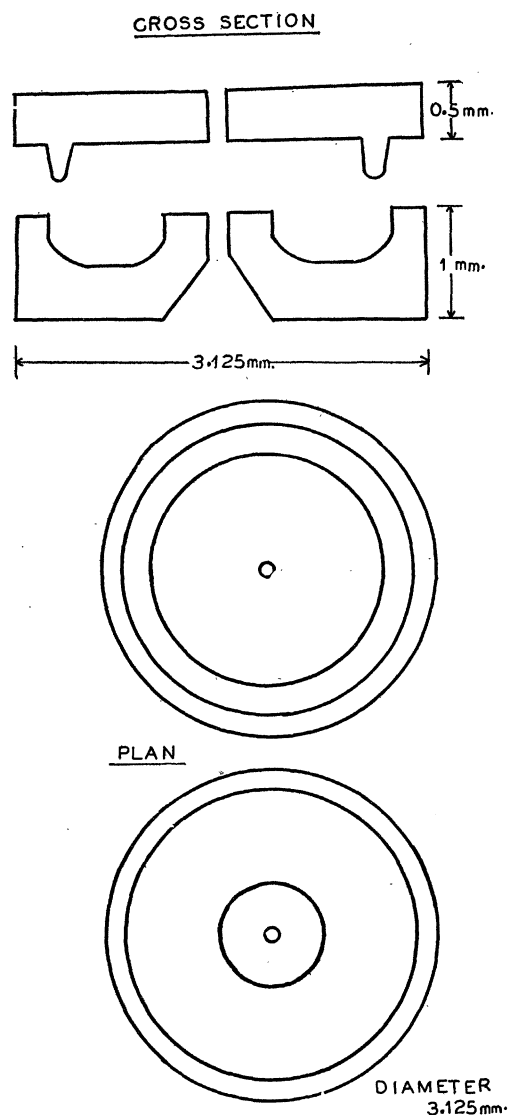


FIG. 1. Perforated platinum discs used to form enclosed electron microscope cell; cell and cover shown in cross section and in plan.

the shell Development Company, Emeryville, for their very generous cooperation in operating the Stanford microscope and the Berkeley R.C.A. microscope, respectively. Without this, our work could not have been carried out.

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A METHOD FOR CENTRIFUGING AT LOW TEMPERATURE

THE laboratory centrifuge is often employed in the preparation of many biological materials. In a good many cases, especially in enzyme work, it is desirable or necessary to centrifuge at a low temperature. We have adapted a Type 1-SB International Centrifuge to run at a low temperature as described below. The centrifuge is in no way impaired for other regular uses.

The drain in the bottom of the centrifuge case is plugged with a rubber stopper, and small pieces of dry ice are placed on the bottom of the case. The amount of dry ice is determined by the length of time of centrifuging. A 17-inch circle of sheet metal with a 5-inch center hole is inserted in the case and is lodged tightly on the bottom of the case over the dry ice by tamping the metal circle along the outer edge. The centrifuge head is placed in position and the centrifuge is ready for use. It is desirable but not necessary to obtain partial insulation of the centrifuge by covering with several layers of cloth.

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