TABLE 1

PHOSPHATE CONTENT OF FRESH EXCISED TISSUES OF RATS KILLED 10 MINUTES AFTER FEEDING SOLUTIONS CONTAINING 22.3×10^{-3} mM/1 OF RADIOPHOSPHATE

Tissue	Radiophosphate found, mM/L. $\times 10^{-3}$. Isotonic diluents								Means	
	NaCl	Ringer	Ringer	Ringer	Ringer	Ringer	Glucose	Glucose	Controls	Glucose
Stomach	1.00	...	1.76	0.64	0.91	0.90	1.17	1.15	1.04	1.16
Duodenum	5.06	2.36	9.70	4.63	0.93	4.44	3.71	2.83	4.52	3.27
Jejunum	8.63	3.25	...	2.91	3.14	6.54	5.33	4.30	4.89	4.81
Ileum I	2.06	4.07	2.84	4.69	1.01	4.20	6.43	5.67	3.14	6.05
Ileum II	...	1.90	0.77	2.21	0.35	1.89	...	...	1.42	...

nation, by use of the Geiger-Müller counter, was then made of the absorbed amount of radioactive phosphate per gram weight of tissue and calculated to millimoles per liter of absorbed phosphate. Since the absorption of radiation by wet tissue is not great, it was felt that in these preliminary experiments ashing might be omitted.

Individual variations proved to be high. Stomach and intestinal contents varied to a marked degree, although all had been deprived of food for the same period of time. While no effect of glucose on the uptake of phosphate was found, the small number of experiments is not decisive but leads to the tentative conclusion that if the permeability of the gastro-intestinal tract to inorganic phosphate is affected by glucose, the effect is small, probably within 30 per cent. The finding of Erf, Tuttle and Scott,[6] who found that phosphate absorption was significantly increased in mice over a three-day period, relates to the utilization within the epithelial cells, and consequently to the intake of phosphate, but without any necessary increase in permeability of these cells to phosphate.

CONCLUSION

It is tentatively concluded that the presence and the presumable absorption of glucose has no important effect on the permeability of gastro-intestinal cells to phosphate.

J. H. PERRYMAN
R. DE LA MADRID
S. C. BROOKS

DEPARTMENT OF ZOOLOGY
UNIVERSITY OF CALIFORNIA

ANTIGENIC DIFFERENCES BETWEEN THE SPERM OF DIFFERENT INBRED STRAINS OF MICE[1]

EVIDENCE has been obtained of the presence in the sperm of C57 strain and P strain mice of antigens lacking in the sperm of B alb C (Bagg albino) strain mice. The procedure followed is essentially the same as one of the common procedures for the demonstration of blood groups in experimental animals, namely, the injection of cells from individuals of one group or antigenic type into individuals of the same species but of a different group or antigenic type, followed by agglutination tests with the resulting antisera against the injected cells. However, to adapt this method to spermatozoa, two modifications of the blood cell technique are necessary. First, because sperm agglutinins are formed even when sperm are injected into the male producing them,[2] it is necessary to absorb all antisera with sperm of some type other than that against which they are to be tested. Second, because one male does not yield nearly enough sperm for the injections and absorptions, pooling of the sperm of a number of males of the same sperm type is necessary. This is possible in the case of mice because of the existence of numerous inbred stocks all spermatozoa from any one of which may be presumed to be genetically and immunologically identical.

Sperm for injections and absorptions were obtained by killing male mice, dissecting out the vasa deferentia and epididymides, mincing these in Locke's in a watch glass with fine scissors, straining through two fine wire strainers into a filter flask, centrifuging to remove excess fluid, and resuspending in the amount of Locke's desired for injection or in the serum to be absorbed. The mice injected were in every case B alb C females. The sperm used for injection were in most cases from C57 males, in one case from F_1 hybrid males between the C57 black and P strains. The sperm used for absorption were usually B alb C, with a control on the adequacy of the absorption procedure usually provided by a parallel absorption with C57 or F_1 sperm. Injections, varying in different series from about 10,000,000 to 30,000,000 sperm each, were made intraperitoneally in courses of 3 injections on consecutive days followed by 4 days' rest. The number of courses varied from 4 to 7. Animals were killed 7 to 14 days after the last injec-

[6] L. A. Erf, L. W. Tuttle and K. G. Scott, *Proc. Soc. Exp. Biol. and Med.*, 45: 652, 1940.

[1] This investigation has been aided by a grant to the Roscoe B. Jackson Memorial Laboratory from the National Cancer Institute. The writer also takes pleasure in acknowledging the valuable assistance of Miss Helen Poucher and Miss Carol Joos.

[2] S. Metalnikoff, *Ann. Inst. Pasteur*, 14: 577, 1900.

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, ±, +, ++, +++, 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 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-$F_1(C57 \times P)$ sperm serum point to the presence of a second distinguishing antigen or group of antigens in the P sperm.

TABLE 1

Sperm injected	Sperm absorbed with	Sperm tested against			
		$F_1(C57 \times P)$	C57	C	P
C57	C57		0		
Same	C		++++	±	
C57	C		++++	+	+++
$F_1(C57 \times P)$	C	+++	++++	+	+++
Same	C57	+++	±	±	++
Control	C		0	0	0

GEORGE D. SNELL
ROSCOE B. JACKSON MEMORIAL LABORATORY,
BAR HARBOR, MAINE

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 cell[1] 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 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

[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.