

about 9 days after ovulation, showed a positive phosphatase reaction in all the epithelioid cells; as would be expected from the earlier observation. Dr. Arthur T. Hertig very kindly arranged to prepare 3 human corpora lutea obtained at operation. One of these was about 1 day old, the second was about 5 days old, and the third accompanied an embryo of the 8th day, No. 8225 of the Hertig-Rock series, Carnegie Embryological Collection. The granulosa lutein cells were free of phosphatase in all three specimens; in the youngest corpus luteum the theca interna contained the enzyme, but not in the other two. That the latter preparations were not defective is shown by the presence of black deposits in nuclei and in endothelial cytoplasm. From this evidence, it is tentatively concluded that the cells of the theca interna contain phosphatase up to a day or two after ovulation but subsequently lose it.

Thus in six species studied five different conditions were observed with respect to the presence and persistence of alkaline phosphatase in the granulosa and theca interna of the mature follicle and the early corpus luteum. The functional significance of this hormone in the ovary can only be conjectured; it seems likely that it is in some way related to the lipids which are plentiful in the follicle wall and the corpus luteum. The distribution of the lipids, like that of phosphatase, is known to be different in various species. Perhaps the phosphatase takes part in the metabolism of phospholipids. If this guess is correct, the puzzling variety in the distribution of phosphatase in the follicles and corpora lutea of different species, observed by Gomori and confirmed here, may ultimately lead to an explanation of the function of the enzyme in such cells as these, for it offers the possibility of tracing an association between phosphatase and one or another of the various intracellular lipids.

As mentioned above, in the ovary of the sow the theca interna is very sharply distinguishable under the microscope by Gomori's method. This fact has permitted following the fate of the theca cells in an ample series of sows' corpora lutea from ovulation to mid-pregnancy. The present writer,² describing the formation of the corpus luteum of the sow as revealed by ordinary histological stains, stated that as the corpus luteum is organized from the collapsed follicle wall, theca interna cells become scattered among the lutein cells derived from the granulosa and are thus more or less disseminated throughout the fully formed corpus luteum. This interpretation was challenged by Solomons and Gatenby,³ but it is completely confirmed by the Gomori preparations. At the 18th day

of pregnancy, for example, the corpus luteum of the sow is made up of phosphatase-free granulosa lutein cells interspersed with phosphatase-laden theca interna cells. Later in pregnancy the granulosa lutein cells also acquire cytoplasmic phosphatase and the picture becomes less clear.

A detailed account with illustrations will be published later.

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ABSENCE OF GLUCOSE EFFECT ON GASTRO-INTESTINAL PHOS- PHATE ABSORPTION

SINCE the fundamental experiments of Cori,¹ the mechanism by which glucose is absorbed from the digestive system has received considerable attention.^{2,3} Primarily from the experiments with phoridzin,⁴ evidence indicated a mechanism involving the coupling of phosphate forming fructose 1-6 phosphate. By the use of tagged (radioactive) ions we made an attempt to determine the relative permeability of the gastro-intestinal wall to phosphate in the presence of glucose as compared with control solutions.

Radioactive phosphate, ^{32}P , with a half-life of 14.2 days, was supplied by the Radiation Laboratory of the University of California in the form of a phosphate solution of pH 7.35 and whose concentration calculated as Na_2HPO_4 was 0.105M and with an activity of 78 $\mu\text{C}/\text{cc}$. The method of preparation of the phosphate as well as the description of the Geiger-Müller counter has been previously given by Brooks.⁵

METHOD

Laboratory rats were placed on a wire screen without food for two days to insure a comparatively empty gastro-intestinal tract. Experimentally 0.4 cc of the radioactive phosphate diluted with 4.6 cc isotonic glucose was fed by stomach tube. Isotonic NaCl or Ringer's was substituted for glucose in the control group. Ten minutes after feeding the animals were killed by decapitation and quickly autopsied. Small portions of the fundus of the stomach, mid-part of duodenum and jejunum, and the upper and mid-portion of the ileum (Ileum I and II of the table) were excised. The sections were uniformly washed with isotonic glucose just enough to remove the unabsorbed and organic material, dried with filter paper and weighed. A determi-

¹ C. F. Cori, *Jour. Biol. Chem.*, 66: 691, 1925.

² W. Wilbrandt and L. Laszt, *Biochem. Zeitschr.*, 259: 398, 1933.

³ L. V. Beck, *Am. Jour. Physiol.*, 133: p. 210, 1941.

⁴ S. Rapaport, N. Nelson, G. M. Guest and I. A. Mirsky, *SCIENCE*, 93: 88, 1941.

⁵ S. C. Brooks, *Biol. Bull.*, 84: 213, 1943.

² G. W. Corner, *Am. Jour. Anat.*, 26: 117-183, 1919.

³ B. Solomons and J. B. Gatenby, *Jour. Obstet. Gynec. Brit. Emp.*, n.s. 31: 580-594, 1924.

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

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ANTIGENIC DIFFERENCES BETWEEN THE SPERM OF DIFFERENT INBRED STRAINS OF MICE¹

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,

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

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 F₁ 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 F₁ 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 injection.

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² S. Metchnikoff, *Ann. Inst. Pasteur*, 14: 577, 1900.