excretion of sugar in the urine increased, and this in turn, without significant change in nitrogen excretion, caused an increase in the D:N ratio. It is impossible at present to interpret the results other than by the process of conversion of fatty acid to glucose.

An alteration in the amount of acetoacetate excreted after introduction of cocarboxylase suggested studies on the possible intermediates involved in the conversion of fat to carbohydrate. Pigeon breast muscle was found to oxidize pyruvic acid faster when treated with an extract of rat kidney cortex which had been incubated with sodium acetoacetate than when treated in the same manner without the acetoacetate, thus indicating the formation from the acetoacetate of some member of the citric acid cycle.[2]

Further investigation with acetoacetic acid showed an increased citric acid excretion in the urine from diabetic animals fed acetoacetic acid plus cocarboxylase (Figs. I and II). In line with this investigation it was found that citric acid is converted to glucose when cocarboxylase is present, but not without cocarboxylase. Actually a small amount of glucose is formed with cocarboxylase alone, but much more when both citric acid and cocarboxylase are present (Figs. III and IV).

The formation of fat from carbohydrate is therefore demonstrated. The course of the conversion is from acetoacetic acid to citric acid, or another member of the cycle, and thence to glucose. The action of cocarboxylase probably is a complicated one, but its use in the depancreatized dog clarifies the principal steps by which the conversion proceeds. Doubtless the same reactions occur in the normal subject fed high fat diets, but because insulin is present the glucose formed is for the most part immediately oxidized and therefore no change in the R.Q., or at most very small changes, are detectable.[3]

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ALKALINE PHOSPHATASE IN THE OVARIAN FOLLICLES AND CORPORA LUTEA

IN a report on the distribution of alkaline phosphatase in normal tissues, made possible by an ingenious method of demonstrating that enzyme in sections, Gomori[1] called attention to the great variation in the distribution of phosphatase in the mammalian ovary. His brief remarks on the subject suggested the advisability of a more systematic study of the ovary in this respect. Because of Gomori's finding that in some species the theca interna and the membrana granulosa of the follicle differ as to their content of alkaline phosphatase, there seemed to be a possibility of tracing the fate of the theca cells in the formation of the corpus luteum, and thus of contributing to the solution of an old and by no means fully settled question.

The observations summarized herewith were made exactly according to the directions of Gomori in the paper cited, except that the period of incubation was lengthened to 2 hours. Statements as to the presence or absence of phosphatase refer only to the cytoplasm; practically all cell-nuclei contain the enzyme and any differences that may exist between them are not relevant here.

Mature follicles of the domestic pig, from sows in estrus, yield striking preparations; the cells of the theca interna are heavily laden with black granules indicating the presence of phosphatase, whereas the cytoplasm of the granulosa cells is entirely free of the enzyme. This difference persists after ovulation and during the organization of the corpus luteum, as will be explained below. Nearly mature follicles from a bitch in estrus were exactly similar to the sow's follicles.

Mature follicles from a rabbit 9 hours after mating showed, on the contrary, a strong concentration of phosphatase in the granulosa but none in the theca interna. In continuance of this condition, in corpora lutea about 8 hours, 3 days and 5 days old, respectively, the lutein cells (most or all of them derived from the granulosa) were laden with the enzyme. Gomori found no phosphatase in rabbits' corpora lutea, except in endothelial cells, but he did not state the age of the corpora lutea and possibly he studied only retrogressive corpora. Ripening follicles ca. 0.8 mm in diameter, of a guinea pig in estrus, less than 1½ hours after opening of the vaginal closure membrane, showed a heavy black deposit representing phosphatase in the theca interna, and a light deposit in the granulosa. Gomori reported the theca interna of the rabbit negative, but he probably studied only immature follicles. Three days and nine days after ovulation, and in a number of pregnant animals given me by Dr. W. L. Hard, the lutein cells were strongly positive, confirming Gomori's statement.

A recently ruptured follicle of the rhesus monkey, obtained within 24 hours after ovulation, while theca interna and granulosa were still clearly distinguishable, showed a strong concentration of phosphatase in both layers. A corpus luteum from another monkey,

[2] The author is indebted to Mr. John J. Kelly and Mr. Robert E. Gosselin, of the Department of Physiology, for their assistance in this part of the work.

[3] E. E. Hawley, C. W. Johnson and J. R. Murlin, *Jour. Nutrition*, 6:523, 1933.

[1] G. Gomori, *Jour. Cell. and Comp. Physiol.*, 17: 71–81, 1941.

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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[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.

ABSENCE OF GLUCOSE EFFECT ON GASTRO-INTESTINAL PHOSPHATE 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, $_{15}P^{32}$, 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 μC/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.