EXPERIMENTAL BIOLOGY

THE ALKALINE PHOSPHATASE ACTIVITY OF THE TISSUES AND THE PRIMORDIAL GERM CELLS DURING THE EMBRYONIC DEVELOPMENT OF THE MOUSE

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Among the large volume of research devoted to the study of the alkaline phosphatase activity in the cells of mammals, a specific place is occupied by histochemical investigations of this enzyme in those cells of the embryo which are usually called the "primordial germ cells". In these reports [4, 5] and also in certain textbooks [3], it emphasized that high alkaline phosphatase activity is a specific sign of the primordial germ cells, distinguishing them clearly from the remaining tissues of the embryo.

This point of view, of importance in principle, is based, however, on a limited amount of factual evidence, comprising only isolated stages of embryonic development. The alkaline phosphatase activity of the developing reproductive glands of the embryo, and also at early stages of embryogenesis, has not been studied.

We accordingly set out to investigate the alkaline phosphatase content throughout the entire period of intrauterine development of the mouse, starting with the ovum.

EXPERIMENTAL METHOD

The age of the embryos was determined from the moment of impregnation of the female; the interval between each two successive stages of development was one day.

The alkaline phosphatase was detected by Gomori's histochemical method, as modified by V. V. Portugalov [1]. In order to minimize inactivation of the enzyme, the treatment of the material from fixation to incubation in the substrate inclusively lasted no more than 24 hours.

The optimal duration of incubation, which was determined empirically, was 1 hour, but in the case of certain sections of the embryo this period was deliberately lengthened to 16 hours and also shortened to 15-30 minutes.

In each experiment, starting from the blastocyst stage, only half the total number of embryos found in the uterus was used for determination of the alkaline phosphatase, the rest being fixed in Zenker's fluid and, after the usual histological treatment, stained by Feulgen's method for DNA and also with hematoxylin-eosin. This enabled the alkaline phosphatase activity to be compared with certain other properties of the primordial germ cells.

In all 109 embryos were investigated, 53 by Gomori's method. In order to judge the degree of alkaline phosphatase activity, in the experiment consideration was paid in the first place to the intensity of staining of the cell by Gomori's method, using the conventional nomenclature of I. I. Sharov [2], and in the second place to the shortest duration of incubation necessary to show the enzymic action of the phosphatase. As a rule the two criteria agreed with each other.

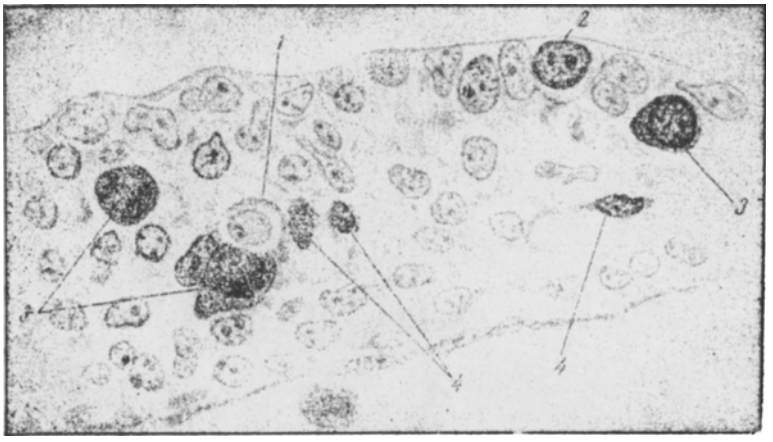

Fig. 1. Area of the germ layer of a 9-day mouse embryo. Stained by Gomori's method. The primordial germ cells (1, 2, 3) show transition from feebly (1) to intensively (3) stained. The individual nuclei of the symplast (4) are also well stained. (The entire figure was produced by means of the Abbe drawing apparatus at the level of the objective table of the microscope under a magnification of 7 × 9).

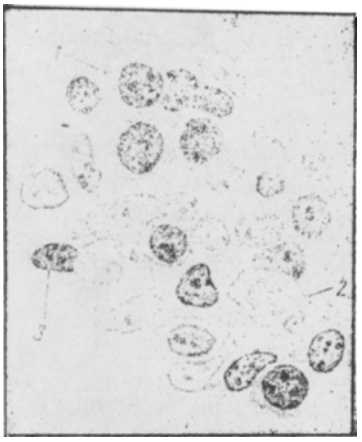

Fig. 2. An area of the undifferentiated sex glands of a 12-day mouse embryo. Alongside the primordial germ cells (1), staining well by Gomori's method, there are others (2) almost unstained. Some nuclei of the symplast are well stained (3).

EXPERIMENTAL RESULTS

The unfertilized oocyte, the polar bodies, zygote, blastomeres and all the cells of the blastocyst contain only traces of alkaline phosphatase, forming a sharp contrast to the tissues of the oviduct or uterus surrounding the embryo. Even after increasing the exposure to 3-4 hours, only the membranes of the cells and nuclei and also the nucleoli were stained.

In the neurula stage (5 days after fertilization) differences in the phosphatase activity were first seen: the neural tissue contained more alkaline phosphatase than the rest of the body of the embryo.

In embryos aged 6-7 days alkaline phosphatase was found in the neural tissue and in the entoderm of the hind gut. In contrast to the neural tissue, the cells of the entoderm were stained unevenly by Gomori's method; transitional stages were seen from cells stained as darkly as the neural cells to others quite lightly stained. The entodermal cells which possessed differing phosphatase activity were in general similar to each other in their dimensions and in the sharp and size of their nucleus, and also in its internal structure and DNA content. The intensity of staining by Gomori's method, even in the darkest of these cells, was much weaker than in the primordial germ cells appearing in the course of the further development of the embryo.

In 8-9 day embryos many organs could be clearly distinguished, but the germ layer was not yet visible. Alkaline phosphatase was present in considerable quantity in all the cells of the spinal cord, in the tissue lining the vesicles of the brain; and in the entoderm of the foregut. A particularly high phosphatase activity, not previously found was shown by individual cells situated mainly in the tissue of the hind gut (ento- and mesoderm), and also in the wall of the abdominal aorta and the umbilical vein (solitary cells). The number of such cells in a section was 1-3. They were arranged singly and haphazardly and some were undergoing division by mitosis; under these circumstances the chromosomes were stained even more intensively than the cytoplasm by Gomori's method.

The tissue of the wall of the gut surrounding the cells, poor in alkaline phosphatase is in the form of a

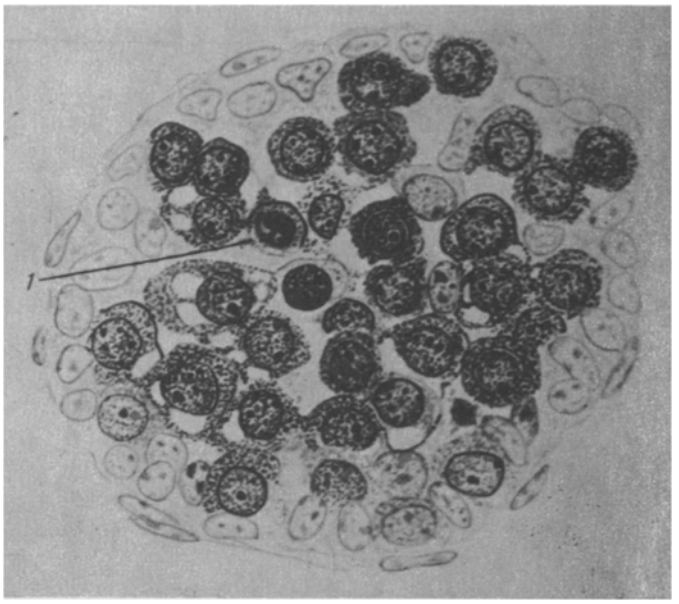

Fig. 3. Transverse section through a testicular cord of a 16-day mouse embryo. Nearly all the primordial germ cells stained intensively by Gomori's method, in contrast to the majority of nuclei of the symplast. 1) degenerating primordial germ cell.

symplast. The structure and staining of the nuclei with hematoxylin and by Feulgen's method were very similar in the cells and the symplast. Some cells were indistinguishable from the symplast in their phosphatase activity, i.e., they contained only traces of the enzyme; between these and the cells staining intensively by Gomori's method there were gradual transitional forms. The individual nuclei of the symplast were also stained intensively, but the cytoplasm belonging to them remained light.

On the 10th day, in many mouse embryos a germinal layer developed on the ventromedial surface of the primordial kidney. When stained with hematoxylin or by Feulgen's or Gomori's method, the tissue of the germ layer and the adjoining mesentery had the same structure as did the wall of the gut at the preceding stage of development. It consisted of a symplast, staining weakly by Gomori's method, and a small number of cells rich in alkaline phosphatase, together with cells which, in their structure and phosphatase activity, appeared to be transitional forms between a symplast and cells (Fig. 1).

In the wall of the aorta, the gut, and the umbilical vein there were also solitary cells containing very much alkaline phosphatase. An average degree of phosphatase activity was observed in the spinal cord and brain and in the optic vesticles; weak activity in the entoderm of the gut. The detritus from dead cells in the lumen of the gut stained well by Gomori's method.

At the undifferentiated gonad stage (11-13th day of intrauterine life) and the first time after the differentiation of the sex (14-15th day) the number of primordial germ cells increased rapidly as the result of their intensive division.

Only a proportion of these cells and many nuclei of the symplast stained by Gomori's method. The intensity of staining of each varied within wide limits (Fig. 2). After prolonged incubation the entire parenchyma of the gland appeared uniformly dark.

Besides the primordial germ cells, therefore, the symplast of the sex gland also possessed a much higher phosphatase activity than the majority of tissues of the embryo. This phenomenon was not due to diffusion of products of cleavage, since the staining was strictly localized in character and did not extend to the tunica albuginea nor to the interstitial tissue of the testis, nor to the tissue of the primordial kidney in immediate contact with it. The germinal epithelium of the testis was darker than the tunica albuginea with any exposure, but lighter than the testis cords.

Around the aorta and the cardinal veins were arranged many irregularly round "islets", characterized by a very high concentration of alkaline phosphatase, even higher than that in the sex glands. Inside a large islet were 10-20 cells and nuclei of a symplast, the small islet consisting of only a few cells. Solitary cells were also encountered which stained darkly by Gomori's method. In ordinary histological preparations, the tissue surrounding the aorta and the cardinal veins contained a considerable number of cells and nuclei of a symplast, arranged singly and in groups and morphologically similar to those in the sex gland. They probably form islets rich in alkaline phosphatase.

Isolated islets were present in the mesentery and the wall of the gut. The majority of islets lay at the level of the gonads, but they were also found outside the limits of this zone in both cranial and caudal directions. This displacement of the islets, and also the presence of a large number of them after completion of sexual differentiation, prevented them from being looked upon as primordial germ cells migrating to the gonad.

At first the spinal cord and brain contained alkaline phosphatase, but its activity fell, and it was not found in all the cells but only in certain areas.

On the following days of intrauterine life (15th-18th days) essential changes took place in the primordial germ cells. Their DNA content was greatly reduced, so that their nuclei were hardly stained by Feulgen's method. They ceased completely to multiple by mitosis, they increased in size and their cytoplasm became vacuolated. Very many cells degenerated.

Nevertheless the alkaline phosphatase activity of the primordial germ cells continued to increase, and the overwhelming majority of them had a very high content of this enzyme (Fig. 3). The contents of the vacuoles did not stain by Gomori's method. The nuclei of the symplast, rich in DNA and multiplying vigorously, now showed (with solitary exceptions) only traces of alkaline phosphatase. No relationship was thus observed between the DNA content and the alkaline phosphatase activity. The degenerating primordial germ cells had the most intensely staining nuclei both by Feulgen's and by Gomori's method.

There was considerable but uneven phosphatase activity in the developing cartilaginous tissue, the perichondrium, and the wall of the ureter (except the mucous membrane). Only traces of phosphatase remained in the spinal cord.

In the last days of intrauterine life a large quantity of alkaline phosphatase appeared in the subcutaneous cellular tissue and in the developing skin glands, coinciding in the second case with an increased quantity of DNA.

There were not, therefore, sufficient grounds for regarding the presence of high alkaline phosphatase activity as a distinguishing feature of the primordial germ cells. Large amounts of this enzyme were also present in other cells of the embryo.

At early stages of the development of the mouse embryo, no cells rich in alkaline phosphatase were observed, and their subsequent formation coincided with the appearance of cells, intermediate in their phosphatase content, in the tissues around them.

SUMMARY

A comparative study of the alkaline phosphatase activity was made in various tissues and primordial germ cells of white mice embryos by using the histochemical method of Gomori, modified by V. V. Portugalov.

Contrary to the commonly accepted opinion it was established experimentally that high alkaline phosphatase activity is an important, but by no means a specific nor strictly constant, property of the primordial germ cells.

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*Original Russian pagination. See C. B. Translation.