CYTOPHYSIOLOGICAL DIFFERENCES BETWEEN THE EMBRYOBLAST AND TROPHOBLAST OF RAT EMBRYOS AS REVEALED BY VITAL STAINING

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The influence of the external environment on the embryogenesis of mammals is usually studied by allowing various agents to act on the maternal organism at different periods of pregnancy. In these conditions it can be very difficult to differentiate strictly between the direct action of the environment on the embryo and the indirect influence of the same factor through the maternal organism. Yet in some cases, for example during the study of the sensitivity of different anlagen, the necessity arises to examine changes in these anlagen brought about by the action of harmful agents at the time or shortly after their application.

For this purpose it is desirable to use cytophysiological methods, notably vital staining of the embryos with neutral red outside the maternal organism after exposure to various agents at different periods of development. However, the correct interpretation of cytopathological phenomena which may be detected by means of a paranecrotic technique using vital staining is impossible without the preliminary study of the pattern of deposition of neutral red as observed in normal embryos.

Little work has been done on the study of mammalian embryos by means of vital staining. According to the available data [9, 10], in rat embryos in the early stages of cleavage (up to 16 blastomeres), the distribution of metachromatic granules in the cytoplasm of the cells varies. This has also been demonstrated by the numerous cytochemical studies carried out in Dalcq's laboratory [4-7, 11, 14-17] and confirmed by the findings of E. A. Pozhidaev [3].

The object of this investigation was to study the phenomena of differentiation in the early stages of development of rat embryos. We were particularly interested to discover what relationship the differences between the blastomeres revealed by vital staining of the ova of rats during cleavage bore to the permanent differentiation of the parts of the embryo appearing after implantation.

EXPERIMENTAL METHOD

The test object consisted of rat embryos in the stages of development from the zygote to the tenth day inclusive. The technique used for staining with neutral red was that described by Nasonov and Aleksandrov [1] and recommended by them for mammalian tissues. A concentration of 1:2000 was found to be most suitable for staining rat embryos at these stages. Embryos of the early stages were obtained by irrigating the oviducts and uterus with Ringer's solution; the ova thus washed out were at once transferred to the dye solution. To study embryos in later stages of development (from the seventh to the tenth day) laporotomy was performed on the pregnant female and the uterus removed and immediately placed in Ringer's solution, where all the subsequent manipulations were carried out. In some cases the embryo was carefully separated from the surrounding decidual tissue under the control of a loupe and transferred whole to the neutral red solution; in this way staining took place from the surface of the embryonic sac. In other cases the wall of the embryonic sac was incised widely but carefully with a razor blade, so that the dye solution came into direct contact with the internal parts of the embryo. In the stages of development after implantation both the isolated embryos and embryos with decidual tissue, retaining their topographical relationships, were stained. Over 100 embryos were studied, with an average of 10 in each stage. The pictures obtained were recorded by photomicrography and the specimens were drawn by means of an Abbe drawing apparatus.

EXPERIMENTAL RESULTS

Staining of the zygote (before fusion of the pronuclei) with neutral red revealed only solitary small granules, scattered uniformly throughout the cytoplasm. Besides very tiny, dust-like inclusions, larger granules were seen. In the stage 2 blastomeres (on the second day of development) granule formation was much more intensive: the size and number of the granules of dye in the cytoplasm of both blastomeres showed an appreciable increase, and they were mainly perinuclear in distribution. In both blastomeres (Fig. 1a) the outlines of unstained nucleoli could be seen, and the nucleus appeared as a translucent spot surrounded by fairly large granules of dye. Tiny granules also were seen scattered throughout the cytoplasm. In one of the blastomeres the number and size of the granules in the perinuclear zone and throughout the cytoplasm were sometimes much larger than in the other. This type of distribution of granules in the stage 2 blastomeres was observed in roughly 50% of experiments (14 of 24 ova). It will be shown later that this may be regarded as the beginning of the cytodifferentiation of the cells of the embryo. Differences in the chemical properties of the first 2 blastomeres in respect of their acid phosphatase content have been observed by Mulnard [12, 13].

With the progress of cleavage the intensity of granule formation in the blastomeres increased. In the stage 4 blastomeres the granules in the perinuclear zone were much larger: they were arranged in the form of large clusters at the side of the nucleus (Fig. 1b). Further, the differences in the intensity of granule formation in the various blastomeres, which was hardly distinguishable in the stage 2 blastomeres, was now much more obvious. It is clear from Fig. 1 that the 2 blastomeres facing upward and to the left contained more granules than those facing downward and to the right.

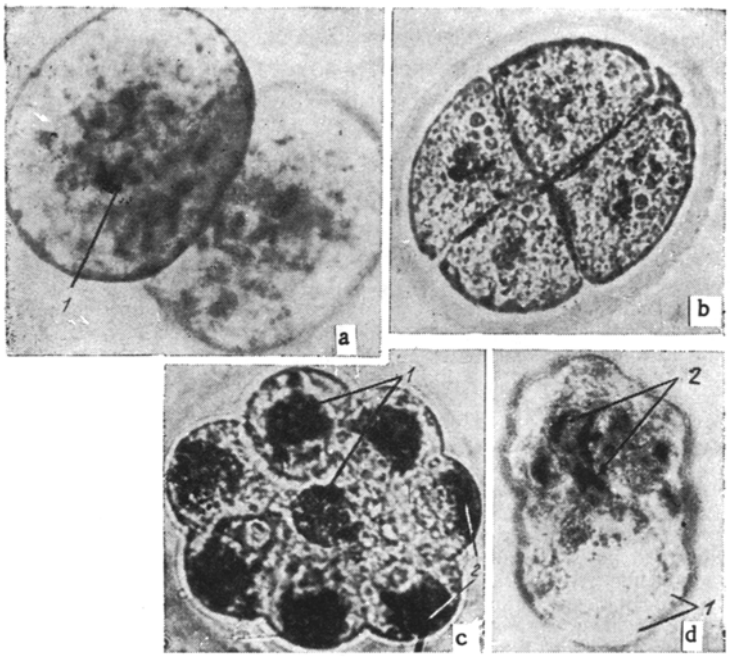

Fig. 1. Granule formation in the period of cleavage of the rat embryo. a) Distribution of neutral red granules in the cytoplasm of the blastomeres on the second day of development (1) more intensive distribution of granules in the perinuclear zone of one blastomere than of the other). Neutral red (1: 2000), objective 40, ocular 10; b) formation of clusters of perinuclear inclusions in the cytoplasm of the blastomeres in the 4 blastomeres stage. Staining and magnification as above; c) stage of 8 blastomeres: distribution of granules of neutral red in the cytoplasm of embryoblastic (1) and trophoblastic (2) blastomeres. Staining and magnification as above; d) embryo of the fifth day of development: formation of blastocyst) more intensive granule formation in the cells of the embryoblast (2) than in those of the trophoblast (1). The same stain. Magnification: objective 20, ocular 7.

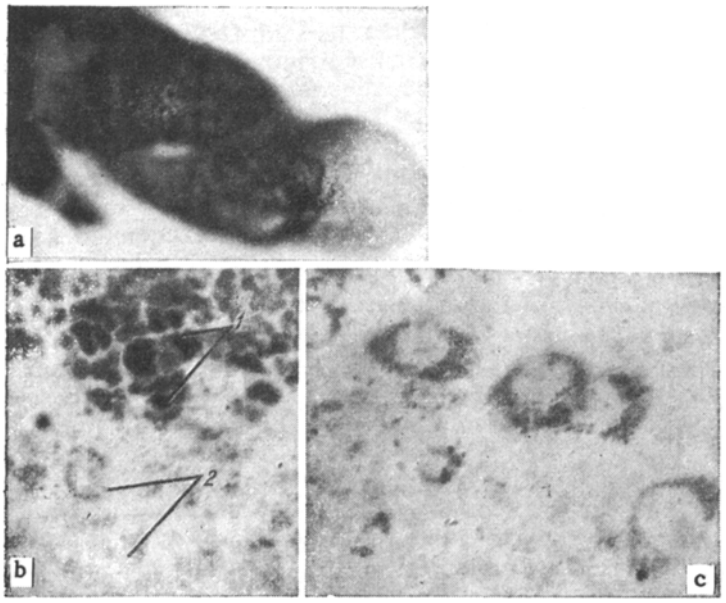

Fig. 2. Granule formation in the cells of an embryo of the tenth day of development. a) Embyronic sac intact: more intensive staining of the cells of the vitelline entoderm and ectoplacental cone (on the left) and much less intensive staining of the cells of the intestinal entoderm (on the right). Neutral red (1 : 2000), objective 10, ocular 7; b) junction of vitelline entoderm (1) and intestinal entoderm (2) of the embryo. Uneven granule formation in the cells of the same anlage. Neutral red (1: 2000), objective 40, ocular 10; c) giant cells of the ectoplacental cone, formation of new granules in the cytoplasm after vital staining with neutral red. The same stain, objective 40, ocular 10.

At the stage of 8 blastomeres (fourth day of development) granule formation was intensified. The number of granules in the perinuclear zone of the blastomeres increased to such an extent that the individual granules merged to form large clusters situated near the nucleus (Fig. 1c). It should be emphasized that the difference in the intensity of staining of the various blastomeres persisted at this stage. Another fact to draw attention was that in some blastomeres granules stained with neutral red were strictly localized around the nucleus, whereas in the others they occupied a peripheral position in the blastomere. This is clearly seen in Fig. 1c, in which an embryo of the fourth day of development is seen from above.

Subsequently the blastomeres with a peripheral arrangement of granules gradually expand to form large blastomeres with numerous granules of neutral red, or, in other words, the former are elements of the trophoblast, and the latter elements of the embryoblast.

On the fifth day formation of the blastocyst takes place, with a more obvious division of the whole cellular material of the embryo into embryo- and trophoblast. The intensity of granule formation in all the cells at this stage of development was slightly less than in the preceding stage. In no case could the large clusters of granules of dye, described above as characteristic of the stage of 8 blastomeres, be found in the embryos of the fifth day. However, in addition to the general fall in granule formation, the difference between the intensity of granule formation in the cells of the embryoblast and trophoblast (Fig. 1d, e) stood out clearly as before. In the cells of the embryoblast large granules of neutral red were present in considerable number and were situated mainly in the perinuclear zone, while in the cells of the trophoblast these granules were smaller and more scattered throughout the cytoplasm.

The study of the distribution of neutral red in the cells of embryos in the early pre-implantation stages of development of the rate thus showed that during cleavage accumulation of neutral red takes place by the formation of new granules, a process regarded by D. N. Nasonov [2] as a nonspecific reaction of living protoplasm to the admission of foreign material. However, the nature of the distribution of the granules of dye at the stages of 4 and 8 blastomeres, when they are arranged in clusters around the nucleus, suggests that certain pre-existing structures are being stained, probably the Golgi apparatus [18, 19, 20].

Immediately after implantation of the embryo further differentiation of the embryo and of the extraembryonic parts of the germinal vesicle took place: the cells of Rauber's layer gave rise to the ectoplacental cone, and later to the trophoblast of the placenta. The yolk sac was finally formed, the mesoderm was formed and began to segment, the intestinal and vitelline entoderm was formed, and the neural tube and allantois began to develop.

After implantation the distribution of dye differed significantly. On the seventh-tenth day of development, when whole germinal vesicles were stained and also after incision of the splanchnopleure dye accumulated most intensively not in the embryonic, but in the functionally specialized cells forming the wall of the embryonic sac. In the first place these were the cells of the extra-embryonic entoderm, the cytoplasm of which was closely packed with intensively stained granules of different sizes. On the tenth day (Fig. 2a, b, c) the difference in the staining of the cells of the vitelline and intestinal entoderm became very obvious. At this stage of development the latter occupies the antimesometric pole of the embryo; it contained hardly any dye, whereas the vitelline entoderm, as which is continued dorsally, was very intensively stained (Fig. 2a). In Fig. 2b and c this difference is shown under high power. Judging by their size and the character of their distribution in the cell, the tiny granules filling the cytoplasm of the cells of the intestinal entoderm were the result of formation de novo. The large droplet-like granules in the cytoplasm of the vitelline entoderm were inclusions, characteristic of the cells of this anlage, stained with neutral red. The giant cells of the trophoblast in the ectoplacental cone were no less intensively stained. On the seventh day of development, when the embryos were stained with the surrounding decidual tissue, the giant cells of the trophoblast, which were distinguishable by their size, were stained much more intensively than the embryonic elements. Large and oftern irregular in shape, they contained a large oval nucleus, and their cytoplasm was filled with tiny, newly formed granules and also with inclusions of considerable size. Hence, besides granule formation, staining of pre-existing structures characteristic of these cells also took place (see Fig. 2c).

The principles of distribution of the granules and the intensity of their development in the embryo- and trophoblastic elements of the rat embryos in the different periods of development may be explained by the peculiarities of growth and differentiation of the various anlagen in mammals in different stages of ontogenesis. The more intensive granule formation in the embryoblastic elements of the embryo during cleavage is apparently determined by the changes in chemical cytodifferentiation of the blastomeres in these stages of development. The intensified granule formation in the tissues of the embryonic sac after implantation of the embryo may result from the fact that at this time the vitelline entoderm and the ectoplacental cone, in contrast to other parts of the embryo, are specialized and functioning tissues.

SUMMARY

The cytophysiological method was used to study the early stages of rat development in vitro. Along with the formation of granules de novo in the cytoplasm of embryonic cells neutral red stained some pre-existing structures. The type of intravital staining and the intensity of granule formation differed at various developmental stages. In the early preimplantation stages (from the second to the fifth day) the intensity of granule formation in the embryoblast cells was greater than in the trophoblast cells. Conversely, immediately after the implantation (the seventh-tenth days) the extraembryonic formations (yolk entoderm, ectoplacental cone, giant cells of the trophoblast) stain more intensely; embryonic and intestinal entoderm stain much more weakly. The character of granule distribution in the tissues of rat embryos at various developmental periods is explained by the peculiarities of growth and differentiation of individual anlagen of mammals at various stages of ontogenesis.

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