THE MITOTIC INDEX AND ACTIVITY OF MITOTIC DIVISIONS IN THE EARLY STAGES OF CHICK EMBRYO DEVELOPMENT

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In the realization of certain morphogenetic processes which depend on the migration of cellular material, unequal tension of cell division in different parts of the embryo may be of considerable significance [4]. It is not surprising, therefore, that many research workers should turn their attention to the investigation of mitotic activity, for example, in the blastoderm of the chick embryo during primitive streak formation [3,6,7,11]. As a measure of mitotic activity, it is usual to use the mitotic index, [m] = Nm/N, i.e., the ratio of the number of mitoses Nm in the total number of cells N, observed in the region under investigation.

Theoretical considerations, however, suggest that the mitotic index is an inadequate measure of the actual activity of cell reproduction [2,11,12]. It is not difficult to demonstrate that, in a cell population which is increasing exponentially and in which cellular reproduction is proceeding by binary division, the mitotic index is given by the following expression

$$[m] = \frac{t_m}{T}\left[N_p\right] l_n 2, \tag{1}$$

where t_m = duration of mitosis; T = generation time; $[N_p]$ = proliferation pool.

Actual mitotic activity may be measured in terms of the rate of population increase per unit time.

$$P = \frac{[\Delta N]}{\Delta t} = \frac{[N_p]}{T} ln2 \tag{2}$$

where $[\Delta N] \cdot 100$ = population increase expressed as a percentage.

From formula (1) and (2), it follows that $[m] = t_m \cdot P$. Thus, the mitotic index can only be an effective measure of cell division activity in such circumstances when the population equals t_m.

In this particular research, we have investigated the actual relationships which exist between the mitotic index, duration of mitosis, generation time, and proliferation activity in chick embryos during early embryogenesis.

EXPERIMENTAL METHODS

We took, as experimental material, chick embryos of the White Leghorn breed at stages III, IV, and V, according to Hamburger and Hamilton [6]. These stages correspond to the middle and definitive primitive streak stages and to the head fold stage. Incubation was carried out in a ZIL-250 M incubator at 38° and at a relative humidity of 50 percent.

In order to determine the mitotic index, we cut transverse sections 6 μ thick and counted 1000 cells or more. All stages of mitosis from early prophase to late telophase were included.

Duration of mitosis was determined by the colchicine method. A solution of colchicine (concentration 10^{-6}) was warmed to 38° and introduced under the blastoderm in amounts ranging from 0.003-0.005 ml. By means of

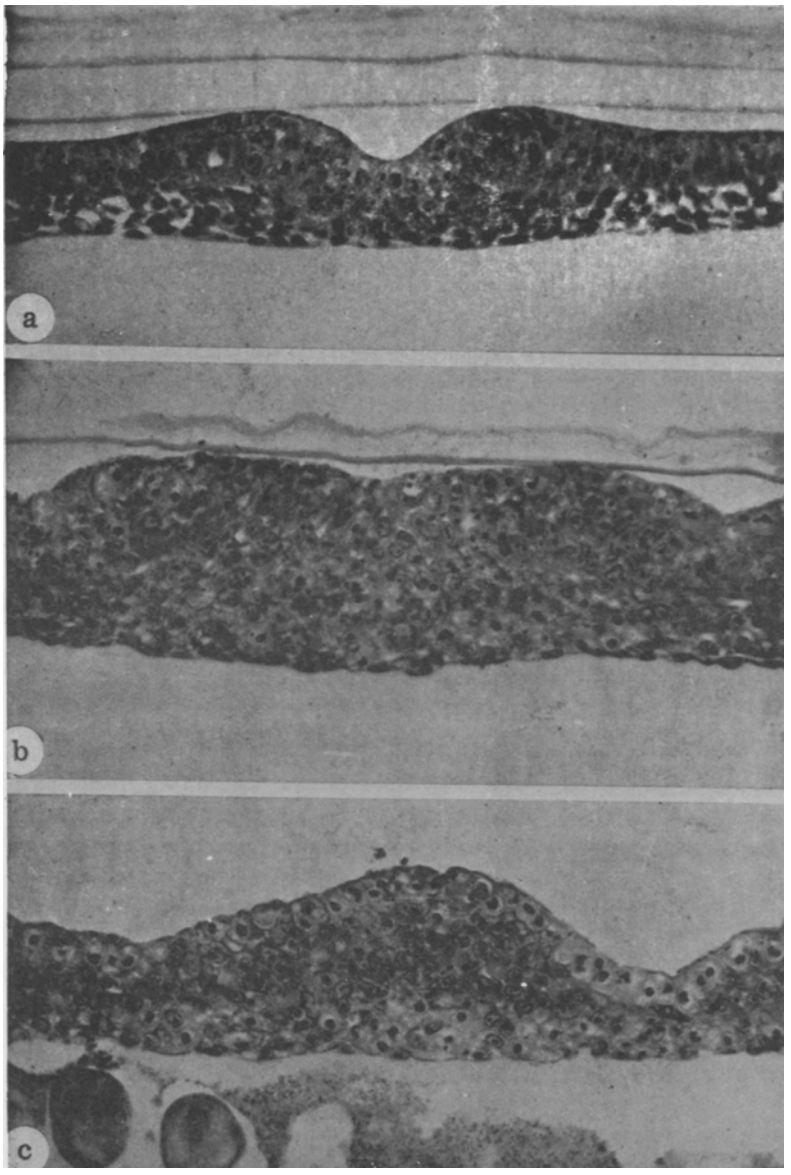

Fig. 1. Transverse sections through the primitive streak of the chick embryo (stage V): a) normal embryo; b) 1 h after colchicine treatment; c) 3 h after colchicine treatment. Ob. × 40; oc × 7. Fixed in Bouin's fluid. Stained with trioxyhematin.

special experiments it had been established that the concentration employed satisfied the requirements of the colchicine technique for these particular stages of embryonic development. Experiments were set up in which the effect of colchicine was continued for 1 h, because after 3 h the normal interrelationships between the parts of the embryo underwent a change (Fig. 1).

The duration of mitosis is calculated from the formula

$$t_m = \frac{[m] \cdot t}{[N_b]}, \tag{3}$$

where $[N_b]$ = index of blocked metaphases; t = time of action of colchicine.

The generation time in populations with a 100% proliferation pool is determined from formulae (1) and (3).

Mitotic Index, Proliferation Activity, Duration of Mitosis, and Generation Time at Various Developmental Stages of Chick Embryos

Zone	Middle streak (stage III)				Definite streak (stage IV)				Head fold (stage III)			
	mitotic index (as %)	proliferation activity (as %/h)	duration of mitosis (in min)	generation time (in h)	mitotic index (as %)	proliferation activity (as %/h)	duration of mitosis (in min)	generation time (in h)	mitotic index (as %)	proliferation activity (as %/h)	duration of mitosis (in min)	generation time (in h)
1	6,8 ± 0,42	8,7 ± 0,44	42	7,9	6,0 ± 0,32	7,6 ± 0,39	47	9,0	7,0 ± 0,13	9,4 ± 0,27	44	7,3
2	8,8 ± 0,44	5,9 ± 0,31	90	11,7	8,4 ± 0,38	6,7 ± 0,34	72	10,1	7,0 ± 0,25	11,7 ± 0,36	35	6,2
3	6,2 ± 0,44	5,7 ± 0,39	60	12,1	6,2 ± 0,36	6,8 ± 0,40	54	10,1	7,5 ± 0,29	10,8 ± 0,40	42	6,3
4	5,4 ± 0,34	9,4 ± 0,51	34	7,3	6,3 ± 0,37	9,2 ± 0,52	41	7,4	9,0 ± 0,32	7,6 ± 0,34	70	9,0
3 (mesoblast)	5,0 ± 0,40	3,5 ± 0,35	90	19,8	4,6 ± 0,38	6,1 ± 0,39	45	11,3	5,5 ± 0,30	9,9 ± 0,37	33	6,9

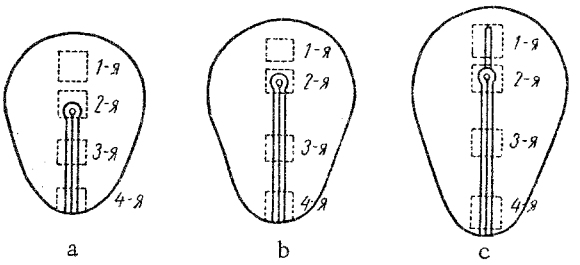

Fig. 2. Investigated zones of blastoderm in the chick embryo: a) stage III; b) stage IV; c) stage V. Various zones indicated by figures.

$$T = \frac{t}{[N_b]} \ln 2. \tag{4}$$

As an index of proliferation activity (P), we took the value which characterizes the rate of population increase per unit time

$$P = \frac{[\Delta N]}{\Delta t} = \frac{[N_b]}{t}. \tag{5}$$

These various indices were investigated in 4 different zones of the ectoblast in the axial region of the embryo and in a single zone of the mesoblast, corresponding to the middle of the embryonic streak.

In stage III embryos, zone 1 corresponds to the area pellucida, zone 2 to the anterior part of the primitive streak, zone 3 to the middle of the streak, and zone 4 to the posterior part of the streak (Fig. 2a). In stages IV and V, zone 1 is the region of formation of the medullary plate, zone 2, the region of Hensen's knot, and the rest correspond with the middle of the streak (Fig. 2, b and c).

EXPERIMENTAL RESULTS

The data obtained is summarized in the table, from which it can be seen that the mitotic index varies from one zone of the embryo to another and varies also according to the particular stage of development reached. Whereas in stages III and IV, the maximum index occurs in the anterior part of the embryonic streak (zone 2), in stage V, it is possible to see a postero-anterior gradient. The lowest mitotic index is associated with the mesoblast at the stage of the definitive embryonic streak.

On comparing the values for mitotic indices with those for proliferation activity, it is obvious that there is no corresponence between the two. In fact, in stage III, the maximum proliferation activity occurs in the posterior region of the embryonic streak, where the mitotic index is considerably lower than at the front end of the streak. The presence of high proliferation activity in zone 4 in each of the subsequent stages of development is confirmatory evidence for the view that the region represents the growth center of the embryo [9, 10].

A high degree of proliferation activity is associated with the zona pellucida (in front of the streak), a region where the medullary plate will form. Attention is drawn to the fact that, at stages III and IV, the region with the maximum mitotic index is characterized by having the lowest

reproductive activity among its cells of anywhere in the whole embryo (zone 2). Furthermore, at the head fold stage, when there is a sharp rise in the proliferation activity of zone 2 (apparently associated with the formation of the head fold itself), the mitotic index falls. This stage is also characterized by a considerable accentuation of proliferation in the mesoderm which may well be related to the accumulation of material necessary for the formation of future somites.

It seems reasonable to suppose that proliferation activity on the part of the cells depends on the speed at which the various stages of the mitotic cycle proceed and on the generation time as a whole. Zones of high proliferation activity are zones with the lowest duration of mitosis and the least generation time. Changes in the duration of mitosis are not always proportional to changes in the generation time. Thus, in the region of Hensen's knot, the duration of mitoses decreases by a factor of 2.6 as the embryo passes from stage 3 to stage 5; over the same period, the generation time decreases by a factor of only 1.9. In the region of zone 4, the duration of mitosis increases by a factor of only 1.9. In the region of zone 4, the duration of mitosis increases by a factor of 2 and the generation by a factor of 1.3 between the same periods.

All this suggests that factors which bring about changes in proliferation activity cannot have exactly the same effect on the various phases of the mitotic cycle.

The generation time in different parts of the embryo undergoes regular changes which can be related not only to the processes of growth but to those of differentiation as well. Thus, at stage III, the generation time among cells of the epiblastic streak is 12 h. However, among those cells which migrate from the epiblast to the interior of the embryo, the generation time is prolonged to a considerable degree (20 h). Later on, the generation time decreases once again in the cells which are mesodermal.

It seems perfectly reasonable to suppose that the sharp increase in generation time mentioned in the last paragraph is related to a definite stage in cell differentiation. Apparently, there is some critical period of change in the differentiation process, which depends on a corresponding change in the rhythm of the mitotic cycle. At the end of this critical period, there may possibly be a new type of cycle.

The method we have employed to determine the generation time presupposes that the cells undergo uniform movements throughout the cycle, thanks to which it is possible to estimate the whole cycle from the rate at which cells enter mitosis. For this reason, any final resolution of the problem of generation time in relation to the process of differentiation as a whole, would require the use of some autoradiographic method, which would permit a direct study to be made of the parameters of the mitotic cycle.

An analysis of the data obtained suggests that there is no real correspondence between the value for the mitotic index and that for proliferation activity. Consequently, the mitotic index cannot be used as any indication of the amount of cell proliferation going on, except where the duration of mitosis in the cell populations under comparison is known to be equal. Hence, the conclusions of those authors who base their estimate of the reproductive intensity of cells on the mitotic index must be suspect.

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