Cell proliferation patterns during cytodifferentiation in embryonic chick tissues: liver, heart and erythrocytes

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SUMMARY

Tritiated thymidine was administered to four groups of embryos at various stages of development. The first group was killed 45 min after isotope administration. The second group received the isotope three times over a 12 h period, the third group four times over a 24 h period, and the last group eight times over a 48 h period. Sagittal histological sections of the embryos were prepared for radioautography. In the radioautographs the percentage labeled nuclei were scored as an index of cell proliferation. The following observations were made.

From stage 10 to 23 (45 min series) there is a progressive increase in the proliferative index of the labeled erythrocytes. This was followed by a precipitous drop as embryonic age increased. At stage 23 of the 12 h series, 97% of the cells were labeled. The 24 and 48 h series of embryos also exhibit a high labeling index, 97% and 95% respectively, by stages 20-24. This indicates that at these stages most of the cells were in the proliferative pool. In correlation with the demonstrated presence of hemoglobin as early as stage 10, it was concluded that cell proliferation and cytodifferentiation in chick primary erythrocytes are not mutually exclusive.

A random pattern of proliferative activity existed in the liver of the early embryos; however, at stage 29 the periphery began to show a higher labeling index than was found in the center. This indicates that liver growth was primarily appositional. From stage 29 through hatching there was a gradual decline in the labeling index. After hatching a burst of proliferative activity occurred but the appositional pattern of growth was not seen. The proliferative activity of the littoral cells remained relatively constant when compared to that of the hepatic parenchymal cells. This suggests that the control of their proliferative activity was somewhat different from that of the hepatic parenchymal cells. The relationship of cell proliferation and cytodifferentiation in the liver could not be established.

There was a progressive increase in the proliferative index of the heart ventricular myoblasts from stage 12 through stage 20-23. This was followed by a gradual decline in proliferation as embryonic age increased. An appositional growth of the heart was also demonstrated. Cytodifferentiation of heart myoblasts is known to occur as early as stage 10. Almost all of the myoblasts were labeled at stages 32 and 33 of the group exposed to the isotope for 48 h. This indicates that, in the heart, cytodifferentiation and cell proliferation are not mutually exclusive processes.

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The purpose of this study is to present a survey of the temporal and spatial patterns of cell proliferation in the erythrocytes, liver and heart of the developing chick embryo. Radioautographic techniques in combination with the specific DNA precursor [³H]thymidine are used in this analysis. These temporal and spatial patterns are then compared with those in the literature.

Over a period of years an embryological dictum stating that 'dividing cells don’t differentiate' has developed concerning the relationship of proliferation to differentiation. This dictum has been reinforced by studies using different cell systems (Doljanski, 1930; Stockdale & Holtzer, 1961; Rumery & Rieke, 1967), while other studies indicate no antagonism between differentiative and proliferative activity (O’Connor, 1953; Hell, 1964; Manasek, 1968; Weinstein & Hay, 1970). The present report gives additional information on this relationship in cardiac muscle, erythrocytes and liver.

MATERIALS AND METHODS

The experimental method of approach involved the use of White Leghorn chick embryos ranging in age from developmental stages 12 to 46 (Hamburger & Hamilton, 1952). Throughout the study the eggs were incubated at 37°C. Tritiated thymidine (1-0 mCi/ml; specific activity 6-7 Ci/mM, New England Nuclear) in doses varying from 0-016 to 0-10 ml, depending on the size of the embryo (0-016 ml for the smallest to 0-10 ml for the largest), was dropped upon the embryo through a window in the shell (Sauer, 1960).

For experimental purposes the embryos were divided into four groups. The first group was sacrificed 45 min after thymidine administration and the second received the isotope three times over a 12 h period. The third set of embryos was exposed to thymidine four times in a 24 h period and the last group eight times in 48 h. The embryos in the 12 h group received the isotope at the beginning of the exposure period, half-way through, and 45 min before the end of the period. Tritiated thymidine was administered to the 24 and 48 h groups every 6 h. The terminal administration of isotope was applied 45 min before sacrifice of the animals in both groups. Variation in the duration of the exposure of the embryos to the isotope enables one to determine both the actual size of the proliferating cell population and the length of time necessary for all of the cells in the various tissues to become labeled. If, after exposure to [³H]thymidine for extended periods, all of the cells in a differentiated population of cells can be shown to be in the proliferative pool, one has evidence that cell proliferative and cell differentiative activities are not antagonistic.

The sacrificed embryos were fixed in Bouin–Hollandé solution. Older embryos were treated by injecting fixative into the peritoneal and thoracic cavities and into the head (brain). Immediately para-sagittal cuts along the length of the embryo...
were made with a scalpel in order to facilitate fixation. The embryos were left in a large volume of Bouin–Holland solution for up to 1 month, which allows decalcification to occur. Embryos up to stage 35 were processed for sectioning using normal histological procedures (Luna, 1968) with the exceptions that amyl acetate was used as a clearing agent instead of chloroform and all paraffin impregnation was done under vacuum. Beyond stage 35, embryos were trimmed of all feathers and the wings and legs removed. Subsequently they were treated according to the processing schedule of hand-processing for the central nervous system (Luna, 1968). Again amyl acetate was substituted for chloroform.

The embryos were sectioned sagittally at 4 μm. One set of slides was stained with Harris’s hematoxylin and eosin. Another set was prepared for radioautography by the dipping technique of Prescott (1964), exposed from 2 days to 4 weeks, developed and stained through the emulsion with Harris’s hematoxylin and eosin. In the radioautographs labeled nuclei were scored as an index of cell proliferation. The labeling index was determined by counting labeled and unlabeled nuclei of cells in a number of arbitrarily chosen areas and is given as the number (or percent) of labeled nuclei per 100 cells. The counting area was defined by means of a Whipple ocular grid. All counts were made using an oil-immersion objective. A minimum of three fields per slide was counted to determine the mean labeling index. In the heart all counts were made in the area of the ventricular apex. All peripheral counts in the heart were limited to an area within 40 μm of the outer surface of the ventricle. In stage 29–33 embryos the innermost counts were limited to an area between 60 and 100 μm from the periphery of the ventricular wall. Because of increased ventricle wall thickness counts in stage 35–39 embryos were made in an area ranging between 80 and 120 μm from the periphery of the ventricle. In each organ care was taken to distinguish cell types, i.e. hepatoblast and littoral cells in the liver, epimyoblast and endocardial cells in the heart, and primary and definitive erythrocytes in the blood. The temporal and spatial aspects of the proliferative activity of two organ systems, heart and liver, and one tissue, erythrocytes, were analyzed.

RESULTS

Erythrocytes

Fig. 1 shows the percentage of labeled primary erythrocytes that are found in the circulation 45 min after administration of [3H]thymidine. At stage 12, 26% of the cells are labeled. A peak in the labeling index of the 45 min series occurs at stages 21–23, where 45% of the primary erythrocytes are labeled. Following this peak a drop in the labeling index to 3% at stage 33 occurs. By the time of hatching (stages 44–46) there are no labeled erythrocytes appearing in the peripheral blood of the 45 min series of embryos (Fig. 1, Table 1). The 12 h series of embryos shows a similar pattern in the proliferative index of the primary erythrocytes. Seventy-four per cent of the erythrocytes at stage 12 and 97% of
those at stage 23 are labeled. Subsequent to this peak of 97\% in the labeling index at stage 23 of the 12 h series, a decline to 0\% at 1 day post hatching (stage 46) occurs (Table 1). The 24 and 48 h series of embryos also exhibit a high labeling index, 97\% and 95\% respectively, by stages 20–24. This peak in the labeling index at about stages 20–24 is followed in each of the two groups by a drop in the number of labeled erythrocytes to 15\% or less by the time of hatching (Fig. 1, Table 1). The erythrocytes shown in Fig. 4A are of the primary series.

![Graph showing the percentage of labeled erythrocytes appearing in the circulation of chick embryos exposed to [\textsuperscript{3}H]thymidine for 45 min. Each point represents one embryo. The curve is a best fit by sight. Abscissa, Hamburger & Hamilton stages of development; ordinate, labeling index percentage.](image)

They show a rounded nucleus and cell shape which is characteristic of the primary erythrocytes. Typically, both the nucleus and cytoplasm of primary erythrocytes stain lightly with hematoxylin and eosin. In contrast, both the cell and the nucleus of the later definitive erythrocytes are oval in appearance with a more intensely staining nucleus and cytoplasm (Figs. 4C, 6D) (Lucas & Jamroz, 1961). Fig. 4A is taken from a stage 29 embryo of the 12 h series in which approximately 37\% of the cells are labeled. Fig. 4B is a photomicrograph of a stage 35 embryo, also of the 12 h series, showing few labeled cells. Both the primary and definitive stages of erythrocytes are present at stage 35; however, most of the erythrocytes occurring here are of the definitive type. Fig. 4C is a picture of the erythrocytes in a stage 44 embryo, also of the 12 h series, in which there are no labeled erythrocytes. All of these cells are of the definitive series.
The embryos were exposed to [3H]thymidine for 45 min, 12 h, and 48 h.

### Table 2. The percentage of labeled myoblasts appearing in the heart of chick embryos at various stages of development

<table>
<thead>
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<th>Length of exposure to isotope</th>
<th>Hamburger &amp; Hamilton stage</th>
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<tr>
<td>45 min</td>
<td>12 13 17 18 20 21 23 24 25 26 27 29 30 32 33 34 35 37 39 40 41 42 44 46</td>
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<tr>
<td>12 h</td>
<td>26 22 33 35 43 43 27 38 24 21 25 6 3 2 2 4 1 . . . 0 7 5</td>
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<td>24 h</td>
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<td>48 h</td>
<td>95 . 94 . 67 73 . 14 .</td>
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* Position of mean labeling index for different embryos at any one stage corresponds to that same position in Tables 1 and 2, thus the value for the top animal at one stage in one table corresponds to the top value at the same stage in another table. A minimum of three counts per slide was made to determine the mean.
Liver

The results of the study of the liver development showed that the spatial distribution of proliferative cells is not uniform throughout the liver during the whole of organogenesis. Prior to stage 29 the proliferative activity of the hepatoblasts is distributed throughout the liver. Fig. 5A taken from a stage 27 embryo of the 45 min series demonstrates the random patterns of labeling in the liver occurring at this time. By approximately stage 29 the periphery of the liver (top line, Fig. 2) began to show more activity than the center (bottom line, Fig. 2),

indicating that growth is primarily appositional. This is illustrated in Fig. 5B, in which about 45% of the peripheral hepatoblasts and 28% of the hepatoblasts in the center of the liver of a stage 35 embryo of the 12 h series are labeled. Statistical treatment of this appositional pattern of proliferative activity in the liver of embryos exposed to the isotope for 45 min was done to test the validity of the pattern. The mean labeling index at both the periphery and center of the liver of stage 29–44 embryos is presented in Table 3. A total of thirty-six counts was made at the periphery and 36 in the center of the liver yielding thirty-six pairs of counts. The peripheral labeling index was higher than that in the center of the liver in thirty-two of the paired counts. In the remaining four paired counts the labeling index in the center was highest. Statistical treatment of the seventy-two

Fig. 2. Graph showing the percentage of labeled hepatic parenchymal cells in the livers of chick embryos exposed to \[^{3}H\]thymidine for 45 min. Each point represents one embryo. The curves are a best fit by sight. \(\bigcirc\), Labeling index percentage prior to beginning of differential growth pattern; \(\bullet\), labeling index percentage at periphery of liver after appearance of differential growth pattern; \(\times\), labeling index percentage in center of liver after appearance of differential growth pattern. Abscissa, Hamburger \& Hamilton stage of development; ordinate, labeling index percentage.
Table 3. The percentage of labeled hepatic parenchymal cells and littoral cells appearing in the liver of chick embryos at various stages of development

The embryos were exposed to [3H]thymidine for 45 min, 12 h, 24 h, and 48 h. A minimum of three counts per slide was made to determine the mean.

<table>
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<th>Embryonic stage</th>
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<th>24 h series</th>
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<td>17</td>
<td>15</td>
<td>28 26</td>
<td>37 37</td>
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(a) Labeling index prior to differential growth, (b) labeling index for periphery of liver, (c) labeling index for center of liver, (d) labeling index for littoral cells.
labeling indices at both the periphery and center of the liver was done using the binomial expansion method for determining probability as follows:

\[
\frac{n!}{p!q!} (0.5)^p (0.5)^q,
\]

where \( n \) equals number of pairs, \( p \) the number of greater values and \( q \) the number of lesser values. The probability that the peripheral labeling index would be greater than the central labeling index in 32 of 36 cases was found to occur by chance much less than one time in 100 (\( P < 0.0000003 \)). As the developmental age of the embryo increases, the proliferative activity concomitantly drops to near zero at the time of hatching in the 45 min (Fig. 2) and 12 h series (Fig. 5C). The 24 and 48 h groups of embryos exhibited the same differentiative pattern of growth as the other series, but with higher activity for both the periphery and center of the liver. Within 1 day after hatching (stage 46) a substantial increase in the proliferative index is recorded. However, the differential pattern of growth does not exist (Fig. 2, Table 3). The decrease in the labeling index of the littoral cells is not as pronounced as that in hepatoblasts during development.

**Heart**

The labeling index of the heart ventricular myoblasts for the 45 min series of embryos is presented in Fig. 3 and Table 2. In each group of embryos the counts were made at or near the ventricular apex. Results for the 12, 24 and 48 h

![Fig. 3. Graph showing the percentage of labeled myocardial cells in the hearts of chick embryos exposed to \(^3\)Hthymidine for 45 min. Each point represents one embryo. The curve is a best fit by sight. Abscissa, Hamburger & Hamilton stages of development; ordinate, labeling index percentage.](image)
Cell proliferation and differentiation

experimental series are included in Table 2. There is an increase in the proliferative index from stage 12 to about stages 20–23. This is followed by a gradual decline to near-zero value at the time of hatching. Immediately following hatching (stage 46) the labeling index again increases (Fig. 3). The 12 h group of embryos exhibits the same general trends of proliferative activity, though with higher values. By stage 23 of the 12 h series, 97% of the epimyocardial cells are labeled. The 24 h embryos showed a high degree of labeling at stage 40 (95%) followed by a gradual decrease to 27% by stage 41. After hatching the labeling index is 15%. The index obtained from the embryos exposed to the isotope for 48 h is 96% and 97% for stages 23 and 24, 98% and 91% for stages 32 and 33, and 68% for stage 41. Essentially all of the cells at stages 20–24 of the 12, 24, and 48 h series as well as those at stage 32 of the 48 h series are in the proliferative pool.

Table 4. Comparison of the mean labeling indices of myoblasts at the periphery and at the inner side of the wall of the heart ventricle of stage 29–39 embryos

<table>
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<tr>
<th>Embryonic stage</th>
<th>Periphery</th>
<th>Inner</th>
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* Mean labeling index at periphery.
† Mean labeling index of inner area.

Photographs of the 12 h series indicate that the proliferative activity in the embryonic heart ventricle occurs at the periphery. Fig. 6 A (stage 29) shows most of the activity to be at the periphery of the ventricular myocardium. In order to validate the appositional pattern of proliferative activity, stage 29, 30, 33, 35, and 39 embryos from the 45 min series were used. The mean labeling index of both the peripheral and innermost counts is presented in Table 4.

A total of fifteen peripheral and fifteen innermost counts were made in the ventricular myocardium yielding fifteen pairs of counts. The peripheral labeling index was higher than that along the inner portion of the wall in each of the fifteen paired counts.

This pattern of activity in the ventricular myocardium was treated statistically, using the binomial expansion method for determining probability as previously described. The probability that the labeling index at the periphery of the ventricular wall would be greater than that along the inner portion of the wall in fifteen of fifteen cases was found to occur by chance less than one time in 100 ($P < 0.004$). There is very little proliferative activity in the interventricular septum at stage 29 and that in the trabeculae carnea seems to be on the endocardium. At stage 35 (Fig. 6 B, C) peripheral activity still predominates; however,
there is increased labeling in the septum. This coincides with Grohmann's (1961) data for the peak in mitotic activity in the septum. The high degree of proliferative activity at the periphery of the ventricle indicates that growth in the heart as in the liver is primarily appositional. By stage 44 there is little proliferative activity in the ventricular myoblasts (Fig. 6D).

**DISCUSSION**

**Erythrocytes**

The primary series of erythrocytes is morphologically distinct from the later definitive series of erythrocytes (Dawson, 1936; Lucas & Jamroz, 1961). It is also known that cell proliferation of the primary series takes place almost exclusively in the peripheral circulation (Romanoff, 1960; Lucas & Jamroz, 1961). The high labeling index which we found in the peripheral circulation of early embryos confirms these earlier cell-proliferation observations. The disappearance of labeled erythrocytes in the peripheral blood at stage 31 is concomitant with the appearance of the definitive series of erythrocytes, whose erythropoiesis is primarily extravascular (Romanoff, 1960; Lucas & Jamroz, 1961). Failure to see high percentages of labeling in the mature definitive erythrocytes of older embryos is probably due both to the fact that erythropoiesis is extravascular and also to the fact that it takes about 4 days for an appreciable number of immature erythrocytes to develop into mature erythrocytes (Cameron & Kastberg, 1969).

The disappearance of the primary erythrocyte series (Lucas & Jamroz, 1961) follows by 2 days the decrease in the labeling index (Table 1). This may indicate that the life-span of the primary erythrocytes is about 2 days or that a dilution of the number of primary erythrocytes is occurring due to the rapidly expanding population of the unlabeled definitive erythrocytes. It is probable that both of these changes are taking place simultaneously.

Our erythrocyte labeling index of the 45 min series (Fig. 1) agrees with the Dawson (1936) pattern of mitotic activity. Both studies show a rise in the cell proliferation index which reaches a peak on the third or fourth days of incubation. O'Connor (1952) did not find the early increase in mitotic activity and tried

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**Figure 4**

(A) Radioautograph of primary erythrocytes from a stage 29 embryo exposed to \( ^{3}H \)thymidine for 12 h before fixation. The figure shows both labeled and unlabeled erythrocytes. The rounded nucleus and cell shape are characteristic of the primary series of erythrocytes. \( \times 550 \).

(B) Radioautograph of erythrocytes from a stage 35 embryo exposed to \( ^{3}H \)thymidine for 12 h. Both primary and definitive erythrocytes are present; however, most are of the definitive series. Only a few labeled cells are present (arrows). \( \times 607 \).

(C) Radioautograph showing only definitive erythrocytes from a stage 44 embryo exposed to \( ^{3}H \)thymidine for 12 h. Note the dark oval nuclei and oval cell shape characteristic of the definitive erythrocytes. There are no labeled erythrocytes. Arrows indicate labeled cells in the wall of the blood vessel. \( \times 992 \).
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to explain the difference between his work and Dawson's findings by stating that Dawson's findings were faulty because of his use of blood smears, which appeared to O'Connor to give an uneven distribution of dividing cells. Another and perhaps more likely explanation of these differences is that a common method of staging embryos was not used in either of the earlier studies. A more accurate comparison of the results might have been made if both authors had been able to use the Hamburger & Hamilton (1952) method of staging embryos. Both Dawson and O'Connor did, however, find that the mitotic index declined to zero in embryos incubated for 8 days. The labeling index of our 45 min series of chicks also exhibited a decline to near-zero values in embryos incubated for 8 days (stage 34).

Lucas & Jamroz (1961) have shown that none of the definitive series of erythrocytes appear in the peripheral circulation prior to 6 days of incubation (stage 29). Our results show that 97% of the erythrocytes at stage 23 are labeled after 12 h of isotope administration, demonstrating that essentially all of the primary series of erythrocytes, which are the only erythrocytes present in the peripheral circulation at this time, are indeed in the proliferative pool (Table 1). This, in conjunction with Hell's (1964) demonstration of the presence of hemoglobin in the primary erythrocytes as early as 1.5 days of incubation (stage 10), enables us to conclude that in the primary series of erythrocytes the processes of cell proliferation and of cytodifferentiation (as evidenced by hemoglobin presence) are not mutually exclusive.

**Liver**

The proliferative activity in the hepatic parenchymal cells remains constant prior to stage 29 when there begins a gradual decline to near zero at the time of hatching (Table 3). A fall in the mitotic activity beginning about stages 27–28 was reported by O'Connor (1954). In agreement, Lövlie (1959) reports decreasing mitotic activity during this same time. Neither author examined the liver for differential spatial patterns of proliferative activity such as we have described (Fig. 6B).

By stage 44 in the 45 min and in the 12 h series of embryos the labeling index

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**Figure 5**

(A) Radioautograph of liver from a stage 27 embryo exposed to [3H]thymidine for 45 min before fixation. Note that the labeled hepatic parenchymal cells are randomly distributed throughout the liver. × 550.

(B) Radioautograph of liver from a stage 35 embryo exposed to [3H]thymidine for 12 h. Note the non-random distribution of labeled hepatic parenchymal cells throughout the liver. The greater percentage of labeled cells are located at the periphery of the liver. × 152.

(C) Radioautograph of liver from a stage 44 embryo exposed to [3H]thymidine for 12 h. Only a few labeled cells are present (arrow). × 416.
dropped to near zero. However, within 1 day post hatching the labeling index had increased (Fig. 2, Table 3). One possible explanation is that feeding initiated DNA synthesis and mitotic activity in certain organs and tissues of the newly hatched chick (Cameron & Cleffmann, 1964).

During the growth of the embryonic liver the proliferative activity of the littoral cells remains relatively constant when compared to the activity of the hepatic parenchymal cells. However, the proliferative activity of the littoral cells drops somewhat as the chick nears hatching (Table 3). The difference in the patterns of proliferative activity between the hepatic parenchymal cells and the littoral cells indicate that their proliferative activity is under somewhat different control.

Two things make the identification of differentiated hepatic parenchyma cells difficult. They are: (1) the diversity of function of hepatic cells and (2) the lack of morphological markers to indicate the presence of the differentiated state. In addition, not only is it difficult to define differentiation in the liver, but also 12% of the cells in the center of the liver (stage 32, 48 h series) are not in the proliferative pool. These non-proliferating centrally located hepatic cells could possibly account for the functional activity of the liver at this stage of development. Together, these facts make it difficult to know whether or not cell proliferation and cytodifferentiation in the liver are mutually exclusive. However, O'Connor (1953), using glycogen presence as an indicator of the differentiated state, has shown that the percentages of cells containing glycogen were about equal in both the dividing and non-dividing cell populations of the liver. This indicates that in the liver the presence of glycogen is not antagonistic to mitosis.

Heart

The relatively low (10–12%) degree of proliferative activity that occurs in the embryonic myocardium at stages 12 and 13 is in agreement with previous investigators (Sissman, 1966; Stalsberg, 1969). However, the rise to 29%
labeled cells at stage 17 of the 45 min series is not in agreement with Sissman, who reported a labeling index of 10-7 at stage 17 after exposing the embryo to [3H]thymidine for 1 h. This discrepancy between Sissman's data and our data is, as yet, unexplained. Sissman also reported that 'no pattern of anatomical distribution of localized areas with higher or lower radioactive indices along any linear axis or around the circumference of the tube could be defined at any stage'. The figure that Sissman used to illustrate his method for selection of areas for counting did indeed show a difference between the labeling indices of the right and left lateral heart walls. However, the different proliferative pattern presented in that illustration proved to be atypical (N. J. Sissman, personal communication, 1969). Stalsberg (1969), though, did demonstrate definite patterns in the mitotic activity of the heart and heart mesoderm. In a study of the transformation of the straight tubular heart to the more complex looped heart in the embryonic chick she noted that there was a consistent rostro-caudal wave of mitotic activity. She too found no difference in mitotic activity between the right and left sides of the heart.

The general rise in mitotic activity through stage 23 and subsequent decline to below 5 % just before hatching in the 45 min series is in general agreement with Grohmann's (1961) data for mitotic activity in the ventricular myocardium. As in the liver, one possible explanation for the post-hatching increase we found may be that feeding stimulates DNA synthesis and mitosis (Cameron & Cleffmann, 1964).

Differentiation of myoblasts into myocardial cells occurs by stage 10 of embryonic development (Lewis, 1919; Manasek, 1968b). Following stages 10–12 an increase in the proliferative activity in the heart occurs (Table 2, Fig. 3) (Sissman, 1966; Stalsberg, 1969). Stalsberg (1969) posed the question of which cells in the ventricular epimyocardium of the developing heart are involved in the increased mitotic activity after stages 10–12. She proposed two possible answers. First, all of the epimyocardial cells are in the proliferative pool. This would indicate that cytodifferentiation and cell proliferation are not mutually antagonistic. The second proposal assumes that the epimyocardium is composed of a heterogeneous population of cells, part of which remains in a stable non-proliferating differentiated state while the rest of the population consists of undifferentiated stem cells. Our investigation supports the first supposition, that all of the epimyocardial cells are actually in the proliferative pool. Table 3 shows that, after exposure to the isotope for 12 and 24 h respectively, nearly all of the epimyocardial cells of stage 20 and 23 embryos are engaged in DNA synthesis. After 48 h of exposure to the isotope nearly all of the ventricular myoblasts at stage 32 are labeled. Other evidence showing that the differentiated epimyocardial cells are in the proliferative pool after stages 10–12 has come from work using the electron microscope. Cardiac myoblasts containing both mitotic figures and myofibrils have been demonstrated at the ultrastructural level (Rumyantsev & Snigirevskaya, 1967; Manasek, 1968a). This, in addition to Weinstein & Hay's
(1970) demonstration that nuclear DNA synthesis can occur in cells with organized myofibrils, shows that cell proliferation and cytodifferentiation in the embryonic heart are not mutually exclusive.

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