Action of nuclear and cytoplasmic fractions of liver homogenate on liver growth in the chick embryo

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A number of observations have been made during a study of the regulation of organ growth which lead to the conclusion that there is a certain correlation between the density of cell packing and mitotic activity in the tissue (Tumanishvili, 1964, 1965a, b; Tumanishvili & Tabidze, 1962, 1963). The density of cell packing is expressed as the concentration of nuclei (CN), reflecting not only the number of cells in a given tissue volume, but an aspect of interrelation of nuclei and cytoplasm as well. Observations have shown that an increase in mitotic activity always leads to an increase in the concentration of nuclei, and a decrease to a decrease. At the same time an increase in the concentration of nuclei appears to cause suppression of mitotic activity, while a decrease of the nuclear concentration to cause vigorous cell division. Thus mitotic activity and nuclear concentration form a typical circuit with a negative feedback mechanism

\[ M \xrightarrow{+} C_N, \quad M \xrightarrow{-} \]

where M is mitotic activity and CN is the nuclear concentration in the tissue.

It has also been found that the average amount of DNA per nucleus depends linearly and inversely on the nuclear concentration in the tissue. Such a dependence provides constant concentration of DNA in the tissue (see references in Tumanishvili, 1965a, b; 1967b; Tumanishvili et al. 1963, 1964). Hence an increase in the nuclear concentration in the tissue leads to suppression of DNA synthesis and vice versa.

On the basis of these observations we have suggested that the nuclear concentration is one of the main regulating factors of the rate of cell reproduction and DNA synthesis. Certain intercellular interactions are effected by means of definite biochemical intermediates. The observed relationships between nuclear concentration, mitotic activity and intensity of DNA synthesis have suggested

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the hypothesis that one of the substances which suppresses mitotic activity and DNA synthesis is of nuclear origin, while the second which stimulates these processes is stored in the cytoplasm. Theoretical considerations and a comparison with some other theories of intra-tissue growth regulations can be found in previous papers (Tumanishvili, 1965a, b; 1967a, b; Tumanishvili, Kozlova & Salamatina, 1967). The assumptions made mainly concern liver tissue, but their extrapolation at least to tissues with the same type of growth and distribution of mitoses does not pose any important difficulties.

To test these ideas nuclear and cytoplasmic fractions of liver homogenates of adult hens or of chick embryos were injected into intact chick embryos. There are reasons for believing that injected fractions would be specifically localized in homologous tissues, since both intact cells and their structural components possess this ability (Weiss & Andres, 1952; Andres, 1953; Ebert, 1954, 1958; Walter, Allmann & Mahler, 1956; Hudnik-Plevnik, Glisin & Simic, 1959; Ebert & DeLanney, 1960; cf. also Tumanishvili, 1965b).

Such an approach is only valid provided that the fractions isolated from cells do not instantaneously lose their ability to act as in intact cells. Although one cannot be sure that this is so, injections of nuclear and cytoplasmic fractions offer one possibility of experimental verification of these assumptions. The fact that isolated nuclei preserve many of their properties for rather a long time favours such experiments (Allfrey, Mirsky & Osawa, 1957; Scholtissek, 1962).

MATERIALS AND METHODS

In the main series of experiments adult mature hens and cocks were used as donors. In some experiments livers of 11-day-old chick embryos were used. Recipient embryos were 11 days old at the onset of the experiment.

Liver tissue was homogenized in 0.25 M sucrose, 0.0018 M-CaCl₂ solution (the ratio of tissue weight to the volume of solution was 1:5) in a teflon-glass Potter homogenizer at 4 °C. The homogenate was filtered through a double layer of gauze. The nuclei were sedimented by centrifugation at 600–700g for 10 min. The supernatant (cytoplasmic fraction) was decanted. The nuclei were re-suspended in half the initial volume of sucrose and centrifuged in the same way. This procedure was repeated three times. The nuclear fraction was analysed microscopically and at the same time the content of DNA in the fractions was determined. Practically the whole of the DNA was found in the nuclear fraction in the preparations used. Microscopic examination showed that neither fraction usually contained intact liver cells and the nuclear fraction contained only a small number of erythrocytes. The fractions obtained were diluted until they contained equal amounts (5 g/100 ml) by dry weight. A hole was made in the shell of the recipient and in the shell membranes and the fraction was injected immediately beneath the membranes with a micropipette. As the site of an injection did not affect the result, it has not been taken into account. However,
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injections into the air chamber were not made. The amount injected was 0.04 ml, i.e. 2 mg dry weight. The mortality was usually negligible.

The embryos were killed at intervals. The liver was fixed in alcohol–acetic acid mixture and embedded in paraffin after the usual treatment. Sections 5 μ thick were stained with hematoxylin and eosin.

The number of mitoses and nuclei per microscope field was counted in preparations at a magnification of ocular 10 × and objective 90 × in 30 fields in each section. Fields containing large blood vessels were omitted. The number of mitoses per 1000 cells was calculated and taken as an index of mitotic activity. The number of nuclei in the field of vision is an expression of their concentration in the tissue (CN). To determine the actual number of nuclei in the section a coefficient of correction was introduced, taking into account the nuclear diameter (Abercrombie, 1946). Nuclear dimensions were measured and the surface area and volume of nuclei have been calculated. The diameter of the nucleus was measured with an ocular-micrometer, 100 nuclei being measured in each liver. As for our results, only the changes of the surface area and volume of the nuclei were of importance; we raised the diameter (d) of the measured nuclei to the second and third power. Only the mitotic activity and the change in number of hepatocytes were of interest. To distinguish hepatocytes and blood cells and their precursors more easily some preparations were stained with azur-eosine and according to Giemsa.

One of the criteria of growth rate in our experiments was the relative weight of the liver (DL). The latter is the percentage ratio of the liver wet weight to that of the whole embryo. The value DL varies but little in normal embryos of the same age but of different weight. It was found that DL changes rather accurately following changes of mitotic activity in the embryonic liver (Tumanishvili & Tabidze, 1962; Tumanishvili, 1964, 1965b). The changes of DL mostly follow the corresponding changes of mitotic activity, lagging some 2–6 h behind them. The dry weight of the liver of the experimental embryos was also determined.

The DNA content in the embryonic liver was determined after the fractionation procedure of Schmidt & Thannhauser (1945) by means of the spectrophotometric method of Tsanev & Markov (1960) which minimizes the influence of impurities on the results. These impurities usually interfere with the determination of DNA content by other methods. The content of DNA was expressed in mg of DNA phosphorus per 100 g wet weight of liver (mg %).

Cytophotometric determination of the mean concentration of DNA in the nuclei of liver cells of experimental embryos was also performed. The concentration of DNA determined for individual nuclei was integrated as usual over the area of each nucleus. Then the average values of these quantities were obtained. In this investigation Feulgen staining was used followed by photography and photometry by means of a microphotometer MF-4.

All the results were compared with corresponding data for control embryos.
In all the figures the values obtained experimentally are given as a percentage of the corresponding control values.

To test the validity of the results Student's $t$ and Wilcoxon-White (cf. Snedecor, 1957) tests were used. In the cases when the standard error of the mean is not plotted in the figures it implies that the error was too small.

The experiments were divided into the following groups:

Group I (control)—uninjected embryos. We had earlier verified that an injection of 0.25 M sucrose solution and of erythrocytes and blood serum of adult hens into chick embryos did not cause any changes of the parameters studied.

Group II—embryos injected with the cytoplasmic fraction of adult hen liver homogenate ($Ct_A$).

Group III—embryos injected with the nuclear fraction of adult hen liver ($N_A$).

Groups IV and V—embryos injected with the cytoplasmic ($Ct_E$) and nuclear ($N_E$) fractions of 11-day-old chick embryonic liver.

Group VI—embryos injected with the cytoplasmic fraction of adult hen liver and again 36 h later with the same fraction ($Ct_A + Ct_A$).

Group VII—embryos injected with the cytoplasmic fraction of adult hen liver and 36 h later with the nuclear fraction of adult hen liver ($Ct_A + N_A$).

Groups VIII and IX—embryos injected with the cytoplasmic fraction of the adult hen liver and 36 h later with either cytoplasmic ($Ct_A + Ct_E$) or nuclear ($Ct_A + N_E$) fraction of 11-day-old chick embryonic liver.

Group X—embryos injected with the cytoplasmic fraction of adult hen liver and 36 h later with either the cytoplasmic or the nuclear fraction of 11-day-old chick embryo livers isolated from embryos injected with the cytoplasmic fraction of the adult hen liver 36 h before. These experiments will be called $Ct_A + Ct_{ECI}$ (when the cytoplasmic fraction of the embryonic liver was injected) and $Ct_A + N_{ECI}$ (when the nuclear fraction was injected). In Groups VI–X the embryos were fixed and studied after 39 and 42 h, i.e. 3 and 6 h after the last injection of the corresponding fraction. The number of embryos for these experiments will be given in each case separately.

To determine mitotic activity and nuclear concentration in experiments $Ct_A$, $N_A$ and the corresponding control we had 45 embryos in each group for each period of time. To determine DNA content and changes of nucleic size 15 embryos were used in each group for each period of time. Five embryos were used in each group for each period of time for cytophotometric studies. One hundred nuclei per embryo were studied. All determinations were made 3, 6, 12, 18, 24, 27, 36, and 48 h after the injection of the corresponding fraction.
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EXPERIMENTAL RESULTS

Changes in the growth rate of the embryonic liver

The mitotic activity in the liver of control embryos varied from 4.0 to 5.2 per 1000 cells and was on average 4.7 ± 0.2 per 1000 cells. The average number of nuclei per field was 97 ± 1, varying from 92 to 100. During the 48 h of the experiment the weight of the control embryos changed from 4510 ± 148 mg to 7714 ± 114 mg and the average liver weight from 81 ± 2 to 142 ± 3 g. The dry weight of the control livers changed from 14 ± 2 mg to 29 ± 3 mg.

![Graph](https://via.placeholder.com/150)

Fig. 1. Changes in mitotic activity and nuclear concentration in 11-day-old chick embryonic liver after the injection of cytoplasmic fraction CtA. (1, mitotic activity; 2, nuclear concentration) and nuclear fraction Na (3, mitotic activity; 4, nuclear concentration) of the adult hen liver homogenate. The numbers are given in % of the corresponding values obtained for the control. Fifteen embryos were used in each group for each period of time.

Cytoplasmic (CtA) and nuclear (Na) fractions of the liver homogenate affect the rate of cell reproduction of 11-day-old chick embryo liver in two opposite ways: while CtA increases mitotic activity and nuclear concentration in the tissue, Na decreases them. The action of CtA is somewhat stronger than that of Na. At the same time the action of the nuclear fraction is observed a little bit earlier than that of the cytoplasmic one (Fig. 1).

An injection of the cytoplasmic fraction into an embryo causes an increase of the relative weight of its liver (DL). In spite of expectations the nuclear fraction also causes some increase of DL if the decrease on the 18th hour of the experiment is ignored. However, the cytoplasmic fraction caused greater increase of DL than the nuclear one (Fig. 2A). As is seen in Fig. 2B, changes of DL depend
Fig. 2. For legend see opposite page.
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mainly on change of liver weight and not on change in embryo weight. The change of the dry weight of the liver corresponds more or less to the changes of its wet weight and $D_L$ (Fig. 2C). The decrease of the dry weight of the liver 12 h after the injection of nuclear fraction is noteworthy (Fig. 2C).

![Graph A](image1.png)

**Fig. 3.** Changes in mitotic activity, nuclear concentration weight and dry weight of 11-day-old chick embryos during 18 h after the injection of a nuclear extract of the adult hen liver. 3 A: change on mitotic activity (solid line) and nuclear concentration (dashed line); five embryos were used for each period of time. 3 B: change of weight indices of the embryonic liver: (1) $D_L$, (2) wet weight and (3) dry weight of the liver. The numbers are given in % of the corresponding values of the control embryos; 20 embryos were used for each period of time.

It was attempted to substitute an extract of the nuclear fraction for the whole nuclear fraction. The extract was prepared by incubation of the nuclei with 0-9 % NaCl for 1 h at 4 °C. Then the nuclei were precipitated by centrifugation at 600 g for 10 min. The supernatant is effectively free of intact nuclei and was injected into embryos (0-04 ml per embryo). This nuclear extract causes an appreciable decrease of mitotic activity and some decrease in weight (both relative and absolute) of embryonic liver (Fig. 3). Changes of $D_L$ in the experiment $Ct_A + N_A$ are peculiar. In this case $D_L$ of embryos was found to decrease to the value characteristic of the control (Table 1) 6 h after $N_A$ had been injected (42nd hour of the experiment). Apparently the drop of mitotic activity taking place between the injection of the nuclear fraction and the time of fixation must have been especially sharp. Indeed, a

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**Legend for Fig. 2**

Fig. 2. Changes in the liver relative weight ($D_L$), the average dry weight of liver and the average weight of 11-day-old chick embryos after the injection of the cytoplasmic fraction ($Ct_A$) (solid line) and nuclear fraction ($N_A$) (dashed line) of adult hen liver homogenate. 2 A is the change of $D_L$, 2 B is the change in average wet weight of liver (upper) and average wet weight of embryos (lower). 2 C is the change of the average dry weight of liver. The numbers are given in % of the corresponding values obtained for the control embryos. Parallel dashed lines show the average values of deviations from the arithmetic mean of the control. The measurements were made with 30 embryos in each group for each period of time.
Table 1. *D*₉, mitotic activity and nuclear concentration in the liver of 11-day-old chick embryos first injected with cytoplasmic fraction of hen liver, at 3 h (39th hour of the experiment) and 6 h (42nd hour of the experiment) after a second injection either of cytoplasmic (*Ct*ₐ + *Ct*ₐ) or nuclear (*Ct*ₐ + *N*ₐ) fraction of hen liver

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of embryos</th>
<th><em>D</em>₉</th>
<th>Mean wt of embryo (mg)</th>
<th>Mean wt of liver (mg)</th>
<th>No. of embryo</th>
<th>Mitoses per 1000 cells</th>
<th>No. of nuclei per field</th>
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<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>1·88 ± 0·05</td>
<td>6556 ± 60</td>
<td>127 ± 6</td>
<td>10</td>
<td>4·1 ± 0·4</td>
<td>93 ± 1</td>
</tr>
<tr>
<td><em>Ct</em>ₐ</td>
<td>10</td>
<td>2·27 ± 0·04</td>
<td>6820 ± 136</td>
<td>156 ± 7</td>
<td>10</td>
<td>5·4 ± 0·2</td>
<td>86 ± 2</td>
</tr>
<tr>
<td><em>Ct</em>ₐ + <em>Ct</em>ₐ</td>
<td>16</td>
<td>2·17 ± 0·04</td>
<td>7525 ± 83</td>
<td>155 ± 6</td>
<td>5</td>
<td>6·2 ± 0·7</td>
<td>88 ± 3</td>
</tr>
<tr>
<td><em>Ct</em>ₐ + <em>N</em>ₐ</td>
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<td>7440 ± 123</td>
<td>147 ± 4</td>
<td>5</td>
<td>2·3 ± 0·7</td>
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39th hour of experiment

<table>
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<tr>
<th>Experiment</th>
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<th>Mean wt of embryo (mg)</th>
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<td><em>Ct</em>ₐ</td>
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<td>7575 ± 150</td>
<td>192 ± 7</td>
<td>10</td>
<td>4·5 ± 0·5</td>
<td>98 ± 2</td>
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<tr>
<td><em>Ct</em>ₐ + <em>Ct</em>ₐ</td>
<td>34</td>
<td>2·16 ± 0·04</td>
<td>7713 ± 82</td>
<td>164 ± 6</td>
<td>5</td>
<td>9·1 ± 1·2</td>
<td>101 ± 3</td>
</tr>
<tr>
<td><em>Ct</em>ₐ + <em>N</em>ₐ</td>
<td>34</td>
<td>1·92 ± 0·05</td>
<td>7545 ± 60</td>
<td>146 ± 6</td>
<td>5</td>
<td>4·5 ± 0·5</td>
<td>94 ± 2</td>
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</table>

42nd hour of experiment

Table 2. *D*₉, mitotic activity and nuclear concentration in the liver of 11-day-old chick embryos injected with the cytoplasmic fraction of hen liver, and injected after 36 h with either the cytoplasmic or the nuclear fraction of the liver of 11-day-old embryos (experiments *Ct*ₐ + *Ct*ₑ, *Ct*ₐ + *N*ₑ, *Ct*ₐ + *Ct*ₑₑₑ, *Ct*ₐ + *N*ₑₑₑ). Fixation and measurement were 3 h (39th hour of experiment) and 6 h (42nd hour of experiment) after the additional injection

<table>
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<td>6523 ± 140</td>
<td>140 ± 5</td>
<td>5</td>
<td>4·3 ± 0·2</td>
<td>92 ± 1</td>
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<tr>
<td><em>Ct</em>ₐ + <em>Ct</em>ₑₑₑ</td>
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<td>2·04 ± 0·08</td>
<td>6945 ± 140</td>
<td>168 ± 5</td>
<td>5</td>
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<td>96 ± 8</td>
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<td>6133 ± 187</td>
<td>126 ± 4</td>
<td>5</td>
<td>1·8 ± 0·6</td>
<td>89 ± 6</td>
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39th hour of the experiment

<table>
<thead>
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<td>10</td>
<td>4·5 ± 0·5</td>
<td>98 ± 2</td>
</tr>
<tr>
<td><em>Ct</em>ₐ + <em>Ct</em>ₑ</td>
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<td>196 ± 6</td>
<td>5</td>
<td>6·2 ± 0·4</td>
<td>95 ± 1</td>
</tr>
<tr>
<td><em>Ct</em>ₐ + <em>N</em>ₑ</td>
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<td>2·21 ± 0·04</td>
<td>8658 ± 151</td>
<td>191 ± 7</td>
<td>5</td>
<td>4·2 ± 0·1</td>
<td>89 ± 1</td>
</tr>
<tr>
<td><em>Ct</em>ₐ + <em>Ct</em>ₑₑₑ</td>
<td>15</td>
<td>2·04 ± 0·02</td>
<td>8761 ± 158</td>
<td>180 ± 4</td>
<td>5</td>
<td>7·0 ± 0·3</td>
<td>97 ± 2</td>
</tr>
<tr>
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<td>170 ± 5</td>
<td>5</td>
<td>3·7 ± 0·4</td>
<td>91 ± 2</td>
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</table>

42nd hour of the experiment
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decrease of mitotic activity was found 3 h after the injection of \( N_A \) into embryos, i.e. on the 39th hour of the experiment (Table 1).

In the experiment \( Ct_A + Ct_A \) the maximum of mitotic activity takes place on the 42nd hour of the experiment, i.e. 6 h after the second injection of the cytoplasmic fraction (see above). \( D_L \) most probably had no time to increase during this period (Table 1).

As we see, the changes of \( D_L \) in this case correspond to the changes in mitotic activity. In the experiment \( Ct_A + N_E \) in which the nuclear fraction of 11-day-old embryonic liver was injected, the results obtained on the 42nd hour of the experiment were different. As may be seen from Table 2, no changes of \( D_L \) and of mitotic activity occur three hours after an injection of \( N_E \) (39th hour of the \( Ct_A + N_E \) experiment). Some decrease in the nuclear concentration should be noted on the liver tissue of experimental embryos in this case (Table 2). The \( D_L \) of the embryos in the experiment \( Ct_A + Ct_E \) differed significantly from the \( D_L \) of the embryos in the \( Ct_A + N_E \) only on the 39th hour of the experiment, but no difference between them occurred on the 42nd hour of the experiment. It should be mentioned that at both the 39th hour and the 42nd hour of the experiment \( Ct_A + Ct_E \) the mitotic activity was somewhat higher than in the controls.

In the experiment \( Ct_A + N_{ECt} \) the picture is conspicuously different. The material for the preparation of the homogenate was taken on the 36th hour of the experiment when the nuclear concentration in the liver of experimental embryos was usually increased (Fig. 1). On the 42nd hour of the experiment, i.e. 6 h after the second injection of the corresponding fraction, \( Ct_A + N_{ECt} \) had strongly inhibited liver growth in recipient-embryos. The \( D_L \) of this group of embryos (Table 2) dropped below the corresponding value in the control group. In this case the mitotic index is not lower than in the control, but on the third hour after the additional injection of the nuclear fraction (on the 39th hour of the experiment) a decrease in mitotic activity took place (Table 2).

\( Ct_A + Ct_{ECt} \) did not cause increases in \( D_L \) when compared to the embryos injected only with \( Ct_A \), though the mitotic activity in this case is somewhat higher than in the control both at the third and the sixth hour of the experiment (Table 2).

It should be noted that in none of the experiments have we observed any change of the number of reticulocytes or of cells other than hepatocytes.

Changes in the size of embryonic liver nuclei due to injection of cytoplasmic and nuclear fraction of hen liver homogenate

The nuclear concentration does not give any idea how the volume and surface area of liver nuclei change under the influence of the fractions investigated. To elucidate this, the diameters of liver nuclei were measured (Table 3).

\( Ct_A \) caused a distinct decrease of the diameter of nuclei from the very beginning of the experiment (3rd hour). The diameter remained smaller than that of
the controls during almost the whole experiment (Table 3), though not always significantly.

\( N_A \) at first (at the 3rd and 6th hour of the experiment) leads to a considerable increase of the average diameter. Then a sharp drop takes place (18th hour) and after that (at the 36th and 48th hour) the diameter increases again (Table 3).

Table 3. Change in the average diameter of the liver nuclei in 11-day-old chick embryos after an injection of cytoplasmic (\( C_tA \)) or nuclear (\( N_A \)) fraction

<table>
<thead>
<tr>
<th>Hours after injection</th>
<th>Control diameter (( \mu ))</th>
<th>( C_tA ) diameter (( \mu ))</th>
<th>( N_A ) diameter (( \mu ))</th>
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<tbody>
<tr>
<td>3</td>
<td>4.00 ± 0.02</td>
<td>3.60 ± 0.09</td>
<td>4.54 ± 0.10</td>
</tr>
<tr>
<td>6</td>
<td>3.97 ± 0.10</td>
<td>3.86 ± 0.05</td>
<td>4.61 ± 0.09</td>
</tr>
<tr>
<td>12</td>
<td>3.88 ± 0.06</td>
<td>3.74 ± 0.10</td>
<td>3.79 ± 0.18</td>
</tr>
<tr>
<td>18</td>
<td>3.98 ± 0.12</td>
<td>3.56 ± 0.01</td>
<td>3.58 ± 0.07</td>
</tr>
<tr>
<td>24</td>
<td>3.66 ± 0.07</td>
<td>3.86 ± 0.09</td>
<td>3.68 ± 0.08</td>
</tr>
<tr>
<td>27</td>
<td>3.98 ± 0.01</td>
<td>3.77 ± 0.06</td>
<td>3.89 ± 0.05</td>
</tr>
<tr>
<td>36</td>
<td>3.96 ± 0.05</td>
<td>3.78 ± 0.08</td>
<td>4.08 ± 0.08</td>
</tr>
<tr>
<td>48</td>
<td>3.87 ± 0.02</td>
<td>3.77 ± 0.07</td>
<td>4.10 ± 0.10</td>
</tr>
<tr>
<td>Average: 3.90 ± 0.04</td>
<td>3.73 ± 0.04</td>
<td>3.96 ± 0.13</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Changes in the product of the average diameter of nuclei and their concentration (\( C_N d \)) for 11-day-old chick embryonic liver during 48 h after the injection of the cytoplasmic (solid line) and nuclear (dashed line) fractions of the adult hen liver homogenate.

Calculations show that an injection of hen liver homogenate fractions influences the product of the diameter and the nuclear concentrations (\( C_N d \)) and hence the total surface (\( C_N d^2 \)) and total volume of nuclei (\( C_N d^3 \)) of embryonic liver. Injection of the cytoplasmic fraction causes \( C_N d \) in most cases to be increased while when \( N_A \) is used it is decreased (Fig. 4). We also tried to determine the change of the ratio of the total surface to the total volume of nuclei, expressed as \( C_N d^2/C_N d^3 \). This magnitude might be a parameter determining the rate of release of substance from nuclei. It is easy to see that this ratio algebraically is
equal to $1/d$ and therefore we can judge changes of the ratio $C_N d^2/C_N d^3$ by changes of $d$ (Table 3). This ratio shows on the whole a tendency to increase under the action of the cytoplasmic fraction and to decrease under the action of the nuclear fraction.

It should be noted that the change in the size of nuclei does not, in most cases, compensate for the changes of the concentration of nuclei in the tissue of the embryonic liver. Both the total surface and the total volume of nuclei differ from the control in the same direction as does $C_N$.

**Changes in the DNA content and concentration in liver tissue and nuclei of 11-day-old chick embryos after injection of nuclear and cytoplasmic fraction of adult liver homogenate**

Changes in the DNA content described below could have been caused by a change in the blood supply of the liver. To test this suggestion the number of nuclei belonging to blood elements was counted. It turned out that in the controls they constituted 8 %; 7 % after injection of cytoplasmic fraction and 9 % after that of nuclear fraction. The difference of 1 % was within the error of the arithmetic mean, and negligible.

![Graph](image)

Fig. 5. Changes in DNA content (mg %P) in 11-day-old embryonic liver after injection of the cytoplasmic ($Ct_A$, solid line) and nuclear ($N_A$, dashed line) fractions of the adult hen liver homogenate. Parallel dashed lines show the average spread in the control. The numbers are given in % of the corresponding values obtained for the control embryos. 15 embryos were studied in each group for each period of time.

DNA determination in the liver tissue of experimental embryos was performed concurrently with that of all other parameters. The content of DNA (in mg % of phosphorus) in the liver of control embryos is on average $20 \pm 1$. It may be seen from Fig. 5 that injection into the embryos of $Ct_A$ brought about a stimulation of DNA synthesis in embryonic liver cell nuclei. This was manifested in an increase in the DNA content in the tissue taking place at the 6th and 36th hour of the experiment (Fig. 5) accompanied by a corresponding increase in the DNA
content per nucleus (Fig. 7A). Since the amount of DNA per nucleus on the 6th hour of the experiment is considerably increased, an increase of DNA content in liver tissue at this time did not appear due to the previously noted decrease in liver mass. At other times the amount of DNA per nucleus is somewhat lower than in the controls, but DNA synthesis is promoted even during these periods of time. The fact that the absolute amount of DNA in the whole liver in embryos of the group \( Ct_A \) is increased throughout almost the whole experiment confirms this (Fig. 6).

\( Na \) causes a decrease of the DNA content in embryonic liver. In the series of experiments a decrease of DNA content takes place by 12 h and is somewhat
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more stable than the increase of the DNA concentration under the action of \( C_t A \) (Fig. 5). The decrease of the DNA concentration in the embryonic liver tissue is accompanied by a decrease in its amount per nucleus (Fig. 7A).

A decrease in the DNA content and its amount per nucleus took place also in the experiments \( C_t A + N_A \). We also obtained a decrease in the content of DNA in embryonic liver in the case of \( C_t A + N_{ECI} \). A decrease in DNA content was noticed 3 h after an injection of the nuclear fraction (Fig. 8A). This decrease was accompanied in all cases by a decrease of the mean amount per nucleus (Fig. 8B).

The cytoplasmic fraction in the experiments involving repeated injection turned out to be less active with respect to stimulation of DNA synthesis. However, as cytophotometric investigations have shown, \( C_t A + Ct_{ECI} \) causes an increase in DNA content per nucleus (Fig. 8B).

![Fig. 8. DNA content (in mg %P) (8A) and its amount per nucleus (8B) in the liver of 11-day-old chick embryos injected with the cytoplasmic fraction of the adult hen and 36 h later additionally injected with the cytoplasmic or nuclear fraction of 11-day-old chick embryonic liver. I, control; II, \( C_t A \); III, \( C_t A + Ct_E \); IV, \( C_t A + N_E \); V- \( C_t A + Ct_{ECI} \); VI, \( C_t A + N_{ECI} \). Five embryos were used in each group. The estimations were made on the 39th hour of the experiment.](image)

Cytoplasmic and nuclear fractions also influence the concentration of DNA in nuclei. The former causes an increase and the latter a decrease in concentration (Fig. 7B). Changes in the DNA concentration in nuclei correspond to a certain extent to changes in mitotic activity. Such agreement has been observed until the 18th hour in the \( C_A \) and \( N_A \) experiments as well as all others.

DISCUSSION

Our experiments show that the cytoplasmic fraction of hen liver homogenate (\( C_t A \)) increases mitotic activity in the liver of the 11-day-old chick embryos, while the nuclear fraction (\( N_A \)) decreases it. Changes in mitotic activity were accompanied by corresponding alterations in nuclear concentration.
The data obtained justify the conclusion that in the nuclei of a tissue in the stationary state there is always some amount of a substance suppressing mitoses, while a substance stimulating cell reproduction can be found in the cytoplasm. It seems that these substances are present in tissue homogenates, macerates, etc., and stimulate or suppress the growth of homologous tissues (cf. Paschkis, 1958; Echave Llanos, 1963; Goss, 1964; Bullough, 1965; Tumanishvili, 1965*).

The cytoplasmic and nuclear fractions of hen liver and of the liver of 11-day-old embryos also affected the rate of DNA synthesis and nuclear size, and hence the nuclear DNA concentration. The action of these fractions is in the main opposite to each other. The cytoplasmic fraction increases the rate of DNA synthesis, decreases the size of the nuclei and hence raises the DNA concentration in the nuclei. The nuclear fraction suppresses DNA synthesis and in most cases increases the size of the cell nuclei and reduces the DNA concentration in the nuclei of liver cells of experimental embryos. With respect to the size of nuclei the action of the nuclear fraction is less pronounced than that of the cytoplasmic one. As to DNA concentration, the action of the cytoplasmic fraction is noticeably weaker.

The influence of fractions injected into embryos is quite marked, in spite of the fact that it may to some extent be masked by the action of the embryo's own regulatory systems. The latter would tend to counteract the effect of the introduced material and to restore the stationary state of the tissue.

The action of the growth regulatory systems of embryos is expressed for example in the phenomenon of 'smoothing out', i.e. recovery of the initial value of $D_L$, after induced change (Tumanishvili & Tabidze, 1962; Tumanishvili, 1964, 1965a, b). In the experiments described in this paper the action of growth regulating systems is suggested by some peculiarities of the results (change of the nuclear size in $N_A$ experiments, lagging of DNA synthesis behind the cell division in the case of $C_{st}$, etc.). These may be the consequences of the activity of the embryo's own growth regulatory mechanisms in responding to the effects of experimental treatment.

The nuclear fraction not only inhibited mitotic activity, but it seemed to cause some increase in cytoplasmic volume. While the cytoplasmic fraction causes an increase of mitotic activity and of concentration of nuclei accompanied by an increase in the weight of the liver, in the case of the nuclear fraction a decrease of mitotic activity and $C_N$ is followed after an initial decrease by a small increase in liver weight. However, in the end a decrease of mitotic activity still leads to a decrease of the organ weight, as is seen in the experiments with the repeated injection of fractions.

Another property of the nuclear fraction is rather important. Nuclear activity depends on the state of the tissue from which it is isolated. The growth-inhibiting activity of the nuclear fraction of the 11-day-old embryonic liver is lower than the mitosis-inhibiting capacity of the adult liver nuclei. But growth-inhibiting activity increased sharply when the nuclear fraction is obtained from the embryo-
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nic liver where the nuclear concentration had been increased by a preliminary stimulation of mitotic activity (experiment $Ct_\Delta + N_{ECO}$). Hence the growth-inhibiting activity of nuclei depends directly on their concentration in a tissue.

One might assume that the mechanism of action of the fractions on DNA synthesis and on mitotic activity is the same, and that the length of the S-phase of mitotic cycle or the rate with which cells enter it regulates the rate of cell division. However, there is no quantitative correlation between the amount of DNA in the nuclei and mitotic activity in the experimental embryonic liver. An increased mitotic activity can take place with a decreased amount of DNA per nucleus (cf. Fig. 7A, 12th and 18th hours of the experiment with adult cytoplasmic fraction). DNA synthesis in such cases lags behind the division of cells. Dissociation of the phases of the mitotic cycle takes place, as described also by other authors (Lark, 1964; Graham, 1966). A decrease in the DNA content of nuclei with an increased mitotic activity can take place if the cells stay in G$_2$-phase rather long, and only enter mitosis later, giving the impression of cell division without passage through the S-phase (Gelfant, 1962, 1963, 1966; Brodski, 1966; Maciera-Coelho, Ponten & Philipson, 1966). The details of this process are being studied by us now. It is already possible to state that regulation of DNA synthesis as well as of mitotic activity takes place at different stages of the mitotic cycle in our experiments. Possibly a connecting link between DNA synthesis and mitotic activity itself is the DNA concentration in cell nuclei, the change of which in our experiments often coincides with a change of mitotic activity. The interconnection between these processes will be considered in a separate paper (Tumanishvili & Salamatina, in preparation).

Our experiments have shown that the interdependence of nuclear size and mitotic activity is not unambiguous. Indeed, a decrease of nuclear size can accompany either an increase or a decrease in mitotic activity (cf. Table 3 and Fig. 1).

As to the total size of nuclei, the main contribution to the relevant magnitudes ($C_Nd$, $C_Nd^2$ and $C_Nd^3$) was found to be made by the concentration of nuclei and only in some rare cases by the mean value of nuclear size. Hence at present we cannot state definitely that the concentration of nuclei is the only parameter influencing mitotic activity as we assumed before (Tumanishvili, 1964, 1965a, b, 1967a). Possibly the total surface area or the total volume of nuclei is the important factor.

We did not observe a regular relationship between the nuclear cytoplasmic volume ratio and mitotic activity or DNA synthesis. If we take into account that the nuclear concentration is determined by the volume of cytoplasm, it is clear that changes in nuclear concentration broadly reflect changes in the cytoplasmic volume. Taking this into account, we can conclude that in some cases the mean value of nuclear-cytoplasmic ratio remains constant with changes in mitotic activity, in others it is decreased and, in yet others, increased. Thus $Ct_\Delta$ causes an approximately 10% decrease in nuclear diameter and a corresponding
10% increase in their concentration. At the same time, by the 27th hour of the experiment $Ct_A$ increases the nuclear concentration much more than it decreases the nuclear diameter and hence rather lowers the nuclear-cytoplasmic ratio, while mitotic activity is sharply increased. $N_A$ causes a significant decrease in the nuclear-cytoplasmic ratio (18th hour of the experiment) which coincides with a decrease of mitotic activity, while on the 3rd and 6th hour of the experiment either a constant or a raised nuclear-cytoplasmic ratio corresponds to a decrease of mitotic activity. The same can be said for the DNA concentration in the tissue and its amount per nucleus.

We have therefore come to the conclusion that both mitotic activity and DNA synthesis are determined, to a great extent, by a quantitative ratio of nuclear and cytoplasmic factors, while there is not always a regular connection between these processes and the size ratio of cells.

The data obtained have confirmed completely our assumption of the existence of a factor inhibiting mitoses in the nuclei of liver cells and of one stimulating mitoses in the cytoplasm. The results of our experiments do not support the point of view of Bullough (1965), according to which intracellular regulation of tissue growth is achieved with the participation of one growth-inhibiting substance alone. It is interesting to note that recently both growth-stimulating and growth-inhibiting substances have been found in chick embryo cells (Rubin, 1966a, b).

The data given in this paper generally agree with the theory suggested by us on intratissular regulation of cell production (Tumanishvili, 1965a, b, 1967a; Tumanishvili et al. 1967). Since a predominance of nuclear factors should be created in the tissue with an increase in nuclear concentration and its decrease would lead to a predominance of cytoplasmic factors, change in nuclear concentration can serve as the basis of regulation of mitotic activity and DNA synthesis.

**SUMMARY**

1. 11-day-old chick embryos were injected separately with cytoplasmic and nuclear fractions of chick adult or embryonic liver homogenates. The cytoplasmic fraction causes an increase of liver mitotic activity in recipients, while the adult liver nuclear fraction decreases mitotic activity.

2. The nuclear fraction of 11-day-old chick embryo liver has low mitotic inhibitory activity. If it is isolated from liver of embryos injected earlier with the cytoplasmic fraction of adult liver (which caused an increase in the concentration of nuclei in donor liver), the inhibiting action of the isolated nuclei is found to be high.

3. Nuclear size decreases under the action of the cytoplasmic fraction. An increase in nuclear size is the initial response to the action of the nuclear fraction, but later a temporary decrease of nuclear size takes place.

4. The cytoplasmic fraction of adult liver, being injected into 11-day-old
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chick embryos causes an increase in DNA synthesis in the tissue. The amount of DNA synthesized in 48 h increases. At some periods of the experiment an increase in the DNA concentration in the tissue takes place, which is accompanied by an increase in DNA content. The nuclear fraction acts in the opposite way, decreasing the DNA content in the tissue as well as its amount and concentration in the nuclei.

5. The results obtained confirm the authors’ assumption of the existence of a factor in nuclei which suppresses mitotic activity and DNA synthesis, as well as of a factor existing in the cytoplasm which stimulates mitoses and DNA synthesis.

RÉSUMÉ

Action de fractions nucléaires et cytoplasmiques d’homogénats de foie, sur la croissance du foie de l’embryon de poulet

1. Des embryons de poulet de 11 jours ont reçu des injections séparées de fractions cytoplasmiques et nucléaires d’homogénats de foie de volailles adultes et d’embryons de poulet. La fraction cytoplasmique provoque un accroissement de l’activité mitotique du foie des receveurs, tandis que la fraction nucléaire de foie adulte diminue l’activité mitotique.

2. La fraction nucléaire de foie d’embryon de poulet de 11 jours a une faible activité inhibitrice sur la mitose. Mais si on l’isole du foie d’embryons auxquels on avait préalablement injecté la fraction cytoplasmique de foie adulte, qui provoquait un accroissement de la concentration en noyaux du foie du donneur, l’activité inhibitrice des noyaux isolés devient élevée.

3. La taille des noyaux diminue sous l’action de la fraction cytoplasmique. Un accroissement de la taille des noyaux constitue la réaction initiale à l’action de la fraction nucléaire, mais une diminution temporaire de la taille des noyaux survient plus tard.

4. La fraction cytoplasmique du foie adulte, injectée dans des embryons de poulet de 11 jours, provoque un accroissement de la synthèse de l’ADN dans le tissu. La quantité d’ADN synthétisée en 48 h. augmente. A divers moments de l’expérience survient un accroissement de la teneur et de la concentration en ADN. La fraction nucléaire agit dans la direction opposée, elle diminue la teneur en ADN du tissu aussi bien que sa quantité et sa concentration dans les noyaux.


REFERENCES


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