ACTIVATION OF THE DIVISION AND GROWTH OF CELLS DURING REGENERATION

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One of the characteristic features of the regenerative process is the proliferation and hypertrophy of cells. Cell division takes place both in organs with high mitotic activity and in several organs whose cells have lost their ability to undergo mitosis in the process of ontogenesis. The mechanism of activation of division and growth of cells during regeneration has been little studied until now. We have shown that the occurrence of cell division during regeneration is connected with activation of the cells by protein products of disintegration of tissues. Injection of protein into the animal caused hypertrophy and division of cells in organs in which under physiological conditions mitoses are absent [1, 2]. Activation of growth and cell division by protein or by products of its incomplete decomposition is effected via the nuclein metabolism of the cell [3]. These findings were obtained mainly in studies of regeneration of the liver.

It appeared likely that the same mechanism lies at the basis of regeneration of other organs. In this connection experiments were carried out in which activation of division and growth of cells was studied during regeneration of the salivary glands.

EXPERIMENTAL METHOD

Experiments were carried out on white mice from $2\frac{1}{2}$ to 3 months old. In the first series of experiments about two thirds of the right submandibular salivary gland was extirpated from the animals. As a control, mice

TABLE 1

Changes in the Mitotic Activity and Dimensions of Cells of the Salivary Gland during Regeneration

Group of experiments	No. of animals	No. of mitoses M + m and coefficient K	Probability p	Area in μ^2 (M ± m)			
				cell	t	nucleus	t
Control	4	2,2±0,5; 1,5	—	91,1±2,2	—	17,5±0,54	—
Regenerating gland	6	11,6±2,0; 1,1	0,002	104,0±4,0	2,85	19,3±0,37	9,0
Undamaged gland	6	6,6±1,1; 2,0	0,006	102,9±2,7	3,45	22,1±0,48	6,6
Control	5	0; —	—	100,5±1,7	—	18,0±0,33	—
Regenerating gland	9	2,6±0,4; 1,5	0,000	135,5±2,7	10,8	23,5±0,42	10,0
Undamaged gland	9	2,2±0,4; 1,3	0,000	124,8±2,2	10,0	22,2±0,54	6,0

from the same litter were used, which were kept under identical conditions but not subjected to the operation. The animals were killed at the same time, 3 days after operation. Histological examination of the regenerating (top) and undamaged (bottom) salivary glands was performed. The mitotic activity was determined by counting the number of dividing cells in a constant area (1.65 mm^2) and by calculation of the phase coefficient (the ratio of the first two phases of mitosis and the last two phases). The areas of the transverse section of the cells and of their nuclei were measured by drawing the projections of the cells and by planimetry of these drawings. In both the operated and the undamaged glands 100 cells were measured in each (50 serous and 50 mucous). In each control and each experimental group of animals measurements were made on the glands of 2-3 mice. The mean values of the dimensions of the cells, given in Tables 1-4, are thus based on measurements of 200-300 cells. By analysis of the distribution curves of the measurements of the serous and mucous cells we obtained identical results, and so to simplify the tables and the figures we included in them the combined values for both types of cell. Besides determination of the mitotic activity and the dimensions of the cells, in each experiment determinations

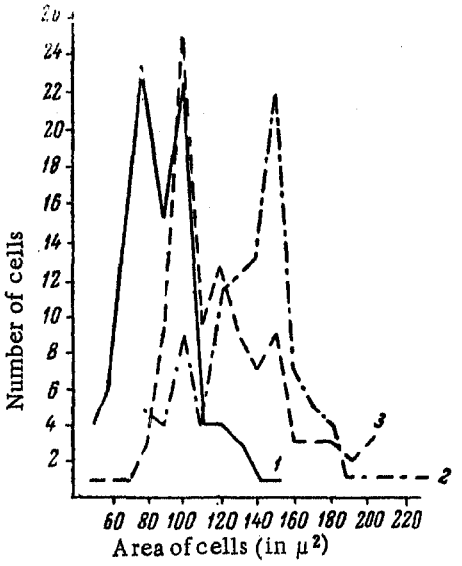

Fig. 1. Distribution curves of measurements of the cells of the salivary glands.

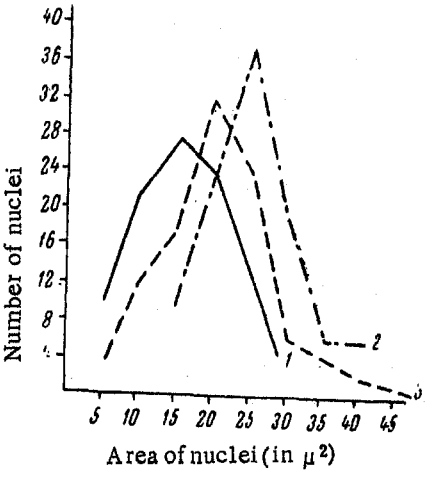

Fig. 2. Distribution curves of the dimensions of the nuclei. Symbols as in Fig. 1.

were made of the ribonucleic (RNA) and desoxyribonucleic acid (DNA) contents of the cell. The material was fixed in Helly's mixture. RNA was detected by staining with methyl green and pyronin by Brachet's method and DNA by the Feulgen reaction.

In the second and third series of experiments, the changes in mitotic activity, dimensions of the cells and DNA and RNA content of the cells of the salivary glands were investigated after injection of protein extracts of various organs. The organ was chopped up finely, extracted with saline for 1-1½ hours in the cold and centrifuged. The extract (0.5-1% of protein) was injected intraperitoneally into white mice in doses of 0.2 cm^3 for 3 days. The material was fixed 3 days after the last injection. In experiments on chick embryos the extract (0.05 cm^3) was injected once only.

EXPERIMENTAL RESULTS

In the salivary gland of the mouse only single dividing cells are found. In many cases in an area of 1.65 mm^2 it is generally speaking impossible to observe mitoses. During regeneration the mitotic activity of the salivary gland increases 3-5 times (Table 1). At the same time an increase in the dimensions of the cells and of their nuclei is observed. Analysis of the curves of distribution (Fig. 1) shows that the increase in the mean dimensions of the cells is connected with a reduction in the number of small cells, with increase in the number of large cells and with the appearance of very large glandular cells which are not met in the control animals.

The number of cells in the classes of average dimensions underwent comparatively little change in the series of experiments. An increase in the dimensions of the nuclei and a clear shift of the distribution curve to the right were also observed (Fig. 2).

Hypertrophy of cells during regeneration of the salivary glands was expressed quite clearly, although to a lesser degree than in regenerating liver. As in other organs, regeneration of the salivary glands was accompanied by a marked increase in the RNA and DNA content of the cells. On the 3rd day after the trauma a marked increase in the intensity of staining of the cytoplasm with pyronin and of the nuclei with Schiff's reagent was observed in the regenerating lobe.

If activation of division and growth of cells during regeneration is connected with a humoral mechanism, it would be expected that it would extend also to the undamaged gland on the opposite side. As can be seen from Table 1 and Figures 1 and 2, the same increase in mitotic activity and in the dimensions of the cells and of their nuclei is observed in the undamaged gland as in the regenerating gland. The intensity of these changes in the undamaged organ is only a little less than in the salivary gland subjected to operation. In the undamaged gland the DNA and RNA content of the cells is also increased. Thus the increase in mitotic activity, hypertrophy of the cells and increase in the DNA and RNA content of the cells extends not only to the regenerating organ but also to the intact analagous organ on the opposite side. The findings which we obtained from a study of regeneration of the liver suggest that activation of the division and growth of the cells of a regenerating organ is connected with the activity of protein products of decomposition of tissue as a result of the trauma. In this connection we carried out a second series of experiments in which healthy white mice were injected with a salivary gland extract. On the 3rd day after a third injection of the extract, the intact salivary glands showed an obvious increase in mitotic activity and in the dimensions of the cells and of their nuclei (Table 2), which was even more sharply expressed than during regeneration (Fig. 3).

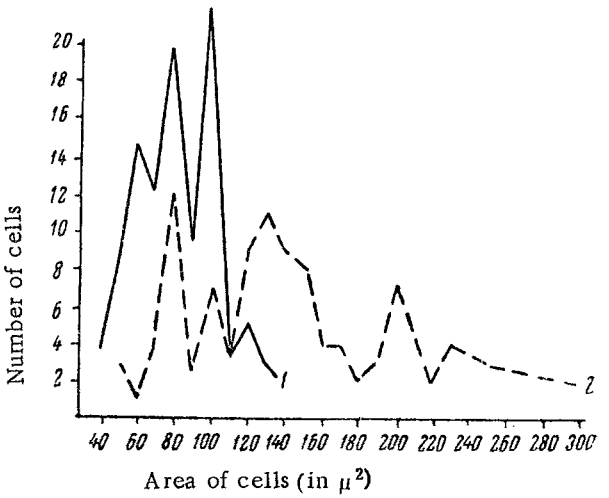

Fig. 3. Distribution curves of the dimensions of the cells of the salivary glands.

Injection of extract, like regeneration, caused a marked increase in the intensity of the reaction to RNA and DNA. After injection of the extract of

Table 2

Changes In Mitotic Activity and the Dimensions of the Cells of the Salivary Gland after Injection of an Extract of this Organ

Group of experiments	No. of animals	No. of mitoses M + m and coefficient K		Probability P	Area μ^2 (M = m)			
					cell	t	nucleus	t
Control	6	0.6±0.2;	—	—	100.8±2.6	—	19.5±0.41	—
Injection of extract	8	5.1±0.4;	3.3	0.000	162.7±4.1	12.8	24.3±0.38	8.6
Control	8	0.1±0.1;	—	—	79.2±1.5	—	19.2±0.33	—
Injection of extract	6	4.1±1.1;	1.0	0.004	126.5±3.3	13.8	26.0±0.48	11.7

salivary gland the mitotic activity in this organ increased more than tenfold. Thus the injection of a protein extract literally reproduces the changes which are observed during regeneration. The identity of these changes suggests that the activation of division and growth of cells observed during regeneration is connected with the activity of protein products of decomposition of tissues as a result of trauma. If the proteins are precipitated (by heating to 60-70°C) the organ extracts lose their ability to stimulate mitotic activity. Processes of proteolysis and the accumulation in the focus of trauma of protein and the products of its incomplete decomposition are, from our point of view, one of the essential factors stimulating the hyperplasia and hypertrophy of the regenerating organ. Since the changes in the RNA and DNA content of the cell precede the stimulation of mitoses and hypertrophy of the cell [3], it can be suggested that activation of cells by protein products of tissue decomposition is effected through the nuclein metabolism of the cell. Data on the role of RNA in the processes of protein syntheses [4, 6-10 and others] and on the importance of nuclein metabolism in cell division [5, 8 and others] make this a likely hypotheses.

Experiments in which various extracts were injected (Table 3) demonstrate the organ specificity of the activating effect of protein on cell division. As can be seen in Table 3, injection of an extract of salivary gland stimulates mitotic activity in this organ only and does not change it appreciably in the epithelium of the cornea

TABLE 3

The Influence of Extracts of Various Organs on Mitotic Activity

Group of experiments	No. of animals	No. of mitoses M ± m and coefficient K					
		salivary gland	P	cornea	P	Intestine	P
Control	8	0.1±0.1; —	—	120±17; 1.0	—	—	—
Extract of salivary gland	6	4.1±1.1; 1.0	0.000	113±12; 1.1	0.922	—	—
Control	6	0.6±0.2; —	—	160±15; 1.1	—	311±19; 1.2	—
Extract of salivary gland	8	5.1±0.4; 3.3	0.000	132±15; 1.1	0.218	281±11; 1.2	0.696
Control	7	0.3±0.1; —	—	62±15; 1.7	—	302±10; 1.7	—
Extract of intestine	7	0.1±0.1; —	0.187	63±19; 1.8	0.922	339±19; 1.5	0.626

or the intestine. At the same time injection of an extract of intestine does not change the mitotic activity in the salivary gland.*

Thus in this series of experiments an organ specificity was apparent in the action of the protein, which we might have surmised on the basis of our experiments on regeneration of the liver[2].

The results of the experiments in which various extracts were injected impelled us to ascertain whether the reaction to injection of protein is preserved in the embryonic period, in conditions when the processes of cell destruction are relatively feebly expressed. An investigation in this direction appeared to be of particular inter-

* Despite the large number of experiments in which the effect of an intestinal extract on the mitotic activity of the epithelium of the duodenum was investigated, we did not obtain any clear results. In the majority of the experiments the mitotic activity did not change and only in individual experiments was it increased to an insignificant degree. Evidently this is related to the high mitotic activity and the intensive mortality of cells occurring in this organ. Probably it is for this very reason that the action of excessive protein does not give here the same effect as is observed in the salivary gland and liver.

est in connection with the findings of a number of workers [7, 11] who demonstrated that injection of protein into an embryo caused an increase in the weight of that particular organ. It seemed likely that these changes are based on the same mechanism of activation of growth and division of cells with which the process of regeneration is connected. We therefore undertook a third series of experiments – on 7-day old chick embryos. Into the vascular field of the embryo was injected 0.05 cm^3 of an extract of the liver of a sexually immature chick of the Leghorn breed. The embryos were fixed 2-3 days after the injection. The ratio of the weight of the liver to the weight of the D embryo, the dimensions of the liver cells and the mitotic activity in the liver were investigated (mitotic activity in an area of 3.3 mm^2).

TABLE 4

The Effect of a Liver Extract on the Division and Growth of the Liver Cells of the Embryo

Group of embryos	D	Area, in μ^2 (M ± m)				No. of mitoses and coefficient K
		cell	t	nucleus	t	
Control	0.0155	88.2±2.5	—	24±0.67	—	143; 1.6
»	0.0101	101.6±2.9	—	24±0.67	—	132; 1.7
Liver extract	0.0223	137.2±4.9	6.9	29±0.87	4.0	204; 3.0
as above	0.0189	131.2±3.5	7.1	28±0.68	3.3	212; 2.9
Control	0.0139	70.4±2.8	—	16.2±0.47	—	71; 1.8
»	0.0112	73.6±1.5	—	16.5±0.51	—	59; 2.2
Liver extract	0.0163	121.8±6.1	7.2	23.2±0.53	8.5	168; 2.5
as above	0.0151	93.6±4.6	3.8	21.4±0.58	5.8	120; 2.5
» »	0.0148	101.4±3.8	5.9	20.7±0.42	5.6	118; 2.2

As can be seen in Table 4, in which are shown the results of two groups of experiments, injection of liver extract causes a relative increase in the weight of the liver. These changes are associated with hypertrophy of the cells and an increase in mitotic activity. Thus even in early stages of development injection of protein causes growth and division of cells. This reaction is organ specific in character. In experiments in which an extract of the spleen was injected we were unable to observe any changes in the mitotic activity or dimensions of the liver cells.

The experiments carried out on chick embryos suggest that activation of mitoses and of growth of cells by protein products of tissue destruction is a phylogenetically primitive reaction of cells to trauma. This is the simplest reaction of the protoplasm, bringing about the stability and preservation of the organism in the midst of the harmful influences of the external environment, and it was evidently established long before the formation of the complex neurohumoral regulation of the growth and division of cells. Death of some cells (or of parts of them) led to the growth and proliferation of others. The experiments of Weisz [15] on the conjoined infusoria (Stentor) provide weighty arguments in support of this point of view. The reaction of cells to the action of protein products of tissue destruction is also preserved in the higher animals as the simplest means of regulation of reparative and, evidently, of physiological [12-14] regeneration. Liberation of protein and the products of its incomplete decomposition lead under these conditions to alterations in the nuclein metabolism and, hence, to growth and division of the cell. The observations described thus suggest that the development of mitoses and of hypertrophy of the cell during regeneration is connected with activation of the cells by protein products of tissue decomposition resulting from trauma.

SUMMARY

An increase of mitotic activity, the size of the cells and their nuclei and the increase of content of RNA and DNA was noted in regeneration of salivary glands in mice. Analogous changes were noted in uninjured gland on the contralateral side. Administration of an extract of salivary gland caused the rise of mitotic activity, an increased content of nucleic acids in the cells and hypertrophy of the cells. Injection of liver extract into the

check's embryo brought about an increase of the relative weight of the liver, the rise of mitotic activity and cellular hypertrophy. A conclusion was drawn that protein and the products of its disintegration possesses an organospecific effect on the growth and mitosis of cells.

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