Stimulating Action of Tissue Extracts on Regenerative Processes

by G. D. TUMANISHVILI

From the Laboratory of Biophysics, Physical Institute of the Georgian Academy of Sciences, U.S.S.R.

WITH TWO PLATES

INTRODUCTION

The problem of regeneration links together the interests of theory and practice very closely, and undoubtedly it is one of the cardinal problems of modern biology. The search for possible methods of controlling regenerative processes, of stimulating selectively the restoration of the main functional components of a tissue, must always lead deeply into the study of the mechanisms of growth and development. The specific influence of intracellular substances on tissue growth and differentiation has been discussed repeatedly. Such substances are sometimes termed ‘tissue organizers’ (Levander, 1945). Levander (1945, 1956) has shown the possibility of forming muscle and bone from poorly differentiated connective-tissue elements either by the transplantation of the corresponding tissues or by the local injection of alcohol extracts of these tissues. Ectopic ossification was obtained in the same way by Lacroix (1945). Distantly and specifically acting ‘stimulators’ have been found in the pulp of rat teeth (Griffié & Velley, 1954). A specific influence of skin extracts on the regeneration of skin has been observed by Marsillii & Ciuti (1953). The liberation from macerates of specifically acting substances that decrease mitotic activity in a homologous organ when it is regenerating has been demonstrated by Saetren (1956) and confirmed by Steuart (see Ebert, 1958b). Others, however, have obtained some increase of the mitotic activity of regenerating liver after injection of liver homogenate into the abdominal cavity (Teir & Ravanti, 1953; Blomqvist, 1957). A stimulating influence of tissue extracts on the regeneration of homologous tissues was also shown by Tumanishvili, Jandieri, & Svanidze (1956b). Saetren himself (1956) obtained a stimulating effect when he used small quantities of macerates.

Such substances also influence embryonic differentiation. The stimulation of growth of an organ homologous to a transplanted one has been observed in chick embryos after transplantation to the chorio-allantois (Murphy, 1916; Ebert, 1954,
1958a) and after injection into the area vasculosa (Weiss, 1947). Increase of mitotic activity in embryonic organs after the injection of suspensions of the corresponding embryonic organs into the blood-vessels of an embryo has also been described (Andres, 1955). Tissue extracts (Tumanishvili, Jandieri, & Svanidze, 1956a) and tissue homogenates (Walter, Allman, & Mahler, 1956) have shown similar effects. A stimulating influence of liver suspensions on the growth of tadpole liver has been found (Romanova, 1957). Probably the mutual influence of paired organs shown by many authors (Weiss, 1952; Saetren, 1956; Liosner, 1958) is due to the same substances.

Existing data are evidently contradictory with respect to the direction of action of the intracellular substances on the homologous structures. Rose (1955, 1958), introducing his concept of 'specific inhibition', thought that specific stimulation does not exist, but the published data hardly allow one to agree with such a view.

The data mentioned above, which I have not space to discuss in full, suggest that substances liberated by cells are important for the regulation of processes which take place in the tissues. Specifically acting intracellular substances are, I suggest, the most direct and primitive means of interaction between homologous cells and of influence by differentiated cells on relatively poorly differentiated cells. These substances may stimulate or suppress homologous cell reproduction and direct the differentiation of less differentiated elements.

The liberation of intracellular substances must proceed especially actively in the initial stage of regeneration because of the intensive cytolysis. This mechanism apparently takes part in the formation of the regenerate, influencing the direction of differentiation of the dedifferentiated tissue elements.

An attempt to use specifically acting intracellular substances to regulate regenerative processes seemed worth making. Since it was assumed that these substances can be transferred through the intercellular medium and blood, they should be extractable with physiological salt solutions. This assumption was confirmed completely in experiments with chick embryos (Tumanishvili, Jandieri, & Svanidze, 1956a). Such extracts have been used in the present experiments with the aim of getting specific stimulation of regeneration. The results have in part been published in preliminary communications (Tumanishvili, Jandieri, & Svanidze, 1956b; Tumanishvili, 1958a).

EXPERIMENTAL METHODS

The effect of tissue extracts on liver and muscle regeneration was studied. The main experiments were made with frogs (Rana ridibunda), but one group of experiments on liver regeneration was made with rabbits and guinea-pigs.

Preparation of the extracts

The extracts in all experiments were prepared in the same way. The tissue was ground thoroughly with quartz sand. For the injections into frogs Ringer
solution or 0.65 per cent. sodium chloride or water was added to the resulting brei. For the injections into mammals the extraction was made with 0.9 per cent. sodium chloride solution. The ratio of tissue-weight to volume of added fluid was 1:2 or 1:1. All these operations were carried out on ice. The mixture was kept for an hour at a temperature of 2–4° C., and then filtered through compressed filter-paper. Such a filter is equivalent to a microbiological filter and the extract so obtained is practically free of cell elements. Membrane filters have recently been used and have given the same results, as also did the removal of suspended particles by centrifugation. The extract may be kept for several days at a temperature not higher than 2° C. By 5–7 days, however, there is an appreciable loss of its stimulating activity.

**Arrangement of injections**

In the experiments with frogs the injections were made post-operatively and then twice a day, every 12 hours. The daily dose at the beginning of the experiment was 0.4 ml., and it was increased by steps till it reached 1 ml. per day. In the experiments on stimulation of liver regeneration the extract was injected for 5 or 10 days, and in the experiments on muscle regeneration for 10, 18, or 27 days. The extract was injected intraperitoneally. In the rabbits and guinea-pigs the injections were made subcutaneously for 10 days, three times a day, 1.5 ml. at each injection with no subsequent increase in dose.

**Stimulation of Muscle Regeneration by Saline Extracts**

The frog gastrocnemius muscle was used in these experiments. A transverse incision into the muscle was made with a scalpel. The length of the incision was about 4 to 5 mm. and the depth about 3 to 4 mm. Because of the contraction of cut fibres and the separation of the wound edges, the wound acquired a funnel-like form, somewhat flattened at the proximal and distal sides.

The frogs were killed after 12, 21, or 30 days, the injections having been made for 10, 18, or 27 days respectively.

There were three main series of experiments: (1) injection of a saline extract of hen muscle (made from pectoral muscle); (2) injection of a saline extract of frog muscle; and (3) injection of a saline extract of hen liver.

There were also control experiments to indicate the course of muscle regeneration in the absence of injection. The results of the experiments are summarized in Table 1. The outcome of the control experiments was very consistent. From the 12th to the 30th day the place of the lesion changed only slightly: it gradually filled with fibrin and structureless masses resulting from the decay of the muscle fibres. No regenerative changes were noticed during this period (Plate 1, figs. A, B). We observed no formation of noticeable groups of myoblasts. However, on the 20th and 30th days one could observe some destruction of fibres in the region of the lesion and their concentration towards the middle of the wound, which might be considered as the beginning of a regenerative process.
When frogs were injected with a hen muscle-extract, the results differed sharply from those of the controls. The wounds were already closed by the 10th day and the lesion was filled with decaying muscle fibres. By the 12th day these changes were still more prominent and there were some bundles of muscle in the lesion. Later, however, they decayed and on the 15th–17th day some nuclei surrounded with a layer of protoplasm emerged from the decaying fibres (Plate 1, fig. E). By

**Table 1**

*Results of experiments on the stimulation of regeneration in frog muscle.*

*Saline extracts unless otherwise stated*

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Post-operative time</th>
<th>Number of specimens</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Hen muscle-extract</td>
<td>20–22</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Hen liver-extract</td>
<td>30</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Frog muscle-extract</td>
<td>12</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Hen liver-extract</td>
<td>20–22</td>
<td>15</td>
<td>14*</td>
</tr>
<tr>
<td>Aqueous extract of hen muscle</td>
<td>30</td>
<td>10</td>
<td>10*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>18</td>
<td>15</td>
</tr>
</tbody>
</table>

* Clearly marked formation of new muscle-fibres was noticed.

the 20th day fully differentiated muscle-fibres and many myoblasts orientated along the future fibres were found in the region of the lesion (Plate 1, figs. C, D, F). A large number of myoblasts was also seen in the region of the lesion on the 30th post-operative day.

Injections of a frog muscle-extract usually did not stimulate regeneration of frog muscles. Closing of the wound edges was noticed in only a few cases. The hen liver-extract showed no significant activity in the stimulation of regeneration.

The fact that the formation of myoblasts is preceded by the phase of fibre destruction, by some concentration of the muscle-fibres inwards towards the lesion, and by filling of the wound, makes us conclude that this phase is a necessary initial stage in muscle regeneration. Such morphological changes in the first phase of muscle regeneration are also described by other authors (Studitski & Striganova, 1951; Rumyantseva, 1956; Samsonenko, 1956). This supposition was also substantiated by the fact that in some of the control samples, especially at later stages, one could notice similar phenomena, though the process proceeded much less actively than in the stimulated wounds. This indicates that there was some acceleration of the regenerative processes under the influences of the extract.
STIMULATION OF LIVER REGENERATION BY SALINE EXTRACTS

These experiments were made mainly with frogs. Frog liver seemed suitable for the experiments as it is known that it regenerates incompletely in winter so that even on the 50th day there is only a connective-tissue scar (Zhenevskaya, 1954). Using spring frogs some investigators got a complete regeneration of the liver in rather a short time (Grigoriev, 1951). Our first experiments were made with winter frogs. Later, however, it was found that within the period of time taken by our experiments there was no special difference between winter and spring frogs, so that it was possible to disregard season (excluding the period of spawning).

The lesion was a hole with a diameter of 0.5 cm. made with a red-hot cylinder. The lesion in the control samples was filled with decaying cell elements and detritus on the 10th–12th day (Plate 2, figs. G, H). The picture in the control samples was very constant, and this gave an opportunity to establish the presence of a stimulating effect in the experiments rather exactly.

Experiments were made on the influence (1) of injected rabbit or hen liver saline extract, (2) of injected frog liver saline extract, (3) of injected hen muscle saline extract. The results are summarized in Table 2.

**Table 2**

**Results of the experiments on stimulation of liver regeneration**

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Post-operative time</th>
<th>Number of specimens</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(days)</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Extract of rabbit liver</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Extract of hen liver</td>
<td>12</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Extract of hen liver</td>
<td>12</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Extract of frog liver</td>
<td>9</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Extract of hen muscle</td>
<td>12</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Aqueous extract of hen liver</td>
<td>9</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Extract of hen liver heated to 60° C. for 5 minutes</td>
<td>12</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

++ Lesion completely filled with liver tissue, or area less than 5–6 mm.$^2$ unfilled.
+ Area of the lesion not filled with liver tissue is less than 15 mm.$^2$
- The area of the lesion not filled with liver tissue is approximately 30–35 mm.$^2$

In a number of cases we measured the area which was not filled with liver tissue. The measurements were made in histological sections taken at different levels from the surface of the liver and an average magnitude characterizing the degree of filling of the lesion was thus obtained. There was a great difference only between the animals injected with rabbit or hen liver-extract and the controls. In the injected animals one could see amitotic cell-division already on the
fifth day, as well as destructive phenomena and some ingrowth of the liver tissue at the edges of the wound. On the ninth day there was much amitotic cell-division and some mitotic cell-division (Table 3), (Plate 2, fig. K), the liver tissue had grown a great deal and only the centre of the wound was filled with structureless masses. By the 12th day in a number of cases the whole lesion was filled with normal liver tissue (Plate 2, figs. I, J) amongst which there were some compact groups of small, apparently recently formed liver cells.

**Table 3**

*Effect of liver extracts on regenerating liver*

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Number of specimens</th>
<th>Number of amitotic divisions per 1,000 cells</th>
<th>Number of mitotic divisions per 1,000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>17.3</td>
<td>7</td>
</tr>
<tr>
<td>Extract of hen liver (saline or aqueous)</td>
<td>5</td>
<td>55.8</td>
<td>21</td>
</tr>
<tr>
<td>Extract of frog liver</td>
<td>2</td>
<td>25.0</td>
<td>10</td>
</tr>
</tbody>
</table>

Calculation of the number of amitotic and mitotic cell-divisions showed that there are appreciably more after injection of hen liver-extract than in the controls (Table 3). The influence of the frog liver-extract was much weaker. We found some slight growth of the liver tissue at the wound edges in some cases, but in most there was no clear difference from the controls. The injection of hen muscle-extract had no influence on the course of the frog liver regeneration.

**Table 4**

*Results of experiments on liver regeneration of rabbits and guinea-pigs*

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Number of specimens</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabbits</td>
<td>Guinea-pigs</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Liver extract</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Liver extract heated to 60°C</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Similar results were obtained in experiments performed with rabbits and guinea-pigs (Table 4). A hole with a diameter of 12 mm. was made in the liver of these animals with a red-hot hollow steel cylinder with a cutting rim. The operated animals were injected subcutaneously with liver-extract of the same species. The injections of 1.5 ml. each were usually made three times a day for 9 days. The animals were dissected 10 days post-operatively. Altogether 19 rabbits and 6 guinea-pigs were operated upon, and of these 6 rabbits and 2 guinea-pigs were used as controls. The control animals had lesions filled with
a friable mass of necrotic cells or, at best, with a connective-tissue scar. In 12 out of 15 cases the lesions of the experimental animals were almost completely filled with liver tissue.

**STIMULATING ACTIVITY OF AQUEOUS EXTRACTS**

In a special series of experiments we tried to stimulate the regeneration of frog liver and muscles with aqueous extract of the corresponding hen tissue. The lesions in liver and muscles were made and the extract was prepared as before. Sodium chloride, or the component salts of Ringer's solution, were added to the filtered extract so as to bring the concentration to 0.65 per cent. of NaCl or to that of Ringer's solution respectively. The extract prepared in this way contains only water-soluble substances.

The experiments showed that aqueous extracts had the same stimulating influence on the regenerative process of frog liver and muscle as the saline extracts (see Tables 1, 2).

**INFLUENCE OF HEAT TREATMENT ON THE STIMULATING ACTIVITY OF THE EXTRACTS**

It was shown in experiments with chick embryos that heating to 60° C. destroyed the stimulating influence of an extract on the homologous organ of an embryo (Tumanishvili, Jandieri, & Svanidze, 1956a). Similar results were obtained with the stimulating influence of extracts on liver regeneration. Hen liver-extract heated to 60° C. for 5 minutes did not stimulate regeneration of frog liver (Table 3). After heating to 60° C., liver extract did not stimulate regeneration of rabbit or guinea-pig liver in spite of the addition of ascorbic acid (a daily dose of 45 mg.) to the extract (Table 2). (It is known that ascorbic acid may accumulate in the liver of rodents. It is also known (Volinski, 1950) that ascorbic acid exerts some stimulating influence on liver regeneration.) Heating an extract to 60° C. always causes coagulation of some of its proteins.

**DISCUSSION**

Our experiments have shown that it is possible to stimulate regeneration of a tissue by an extract of the corresponding tissue. A similar phenomenon was obtained by Korkia (1956) with earthworms. She obtained stimulation of regeneration of the cutaneous-muscular body wall under the action of cutaneous-muscular extract, and she also obtained hyperplastic growth of the circular muscle-layer. The work of this author shows that the influence of tissue extracts on homologous tissue structures cannot be attributed only to mechanical displacement of the tissue components. It is obvious that the effect of the extracts is the stimulation of the regeneration process as a whole, at the basis of which, apparently, lies the increase of specific intracellular syntheses. This hypothesis is also confirmed by the facts which we have obtained. The increase of cell-division
in the liver and the stimulation of the formation of myoblasts in muscles, which take place under the influence of extracts, can hardly be explained in any other way.

The action of extracts on the process of regeneration certainly has an elective tissue-specific character. Unfortunately it is impossible at present to say anything definite about the degree of specificity. However, the negative results of the attempts to stimulate regeneration of liver with a hen muscle-extract, and regeneration of muscles with a liver extract, demonstrate the elective action. The same experiments give reason to exclude the hypothesis that the injected protein extract acts as a nutrient. The nitrogen content of the extracts (determined by the Kjeldahl method) showed that all contained about the same quantity of protein: 35–40 mg. per 1 ml. of extract.

Tissue extracts are apparently characterized by specificity of two kinds: (1) the substances of the extract stimulate the reproduction only of certain cells and accelerate differentiation only in one direction (specificity of action), and (2) the stimulating agent interacts only with the homologous molecular substratum, resulting in its concentration in the homologous tissue (specificity of contact). Indeed, if the stimulating substances should be distributed in all tissues at random they would not so easily achieve an effective concentration in the place of the lesion. Such elective concentration of proteins in chick embryos was demonstrated by Ebert (1954, 1955) and Walter, Allman, & Mahler (1956).

These authors (Ebert made transplantations of organs to the chorio-allantois, and Walter et al. injected tissue homogenate into embryos) used methionine labelled with S\textsuperscript{35}. It was established that proteins containing radioactive methionine moved on the whole into the homologous organ. There is every reason to suppose that an analogous phenomenon takes place in the stimulation of regeneration of any tissue of adult animals.

As already mentioned, some authors observed an inhibiting action of substances isolated from tissues (Steuart—see Ebert, 1955, 1958; Marsillii & Ciuti, 1953; Rose, 1955, 1958; Saetren, 1956; Weiss, 1952). We have never observed such an effect. Within the range of doses used in our experiments the effect always depended directly on the amount of the injected extract. But more recently it has been possible to show (in experiments with chick embryos) that the occurrence of a stimulating effect is closely connected with the quantity of extract injected. Stimulation of growth of the homologous organ takes place only in an optimum dose-range, and with further increase of the quantity injected the stimulating effect disappears completely. But even the largest dose does not depress the growth of the homologous organ (Tumanishvili, 1955). These data contradict the hypothesis of 'specific inhibition' (Rose, 1955, 1958) and also make one rather careful about Weiss's hypothesis of the auto-regulation of tissue growth (1947, 1950). This hypothesis was based on data obtained in experiments with embryos, but it could be applied to regeneration of tissues of adult animals. It is likely that the small mobile extracellular elements to which Weiss ascribed
Inhibiting action would pass into an extract. When the extract is injected one should therefore first notice inhibition of regeneration; but this was not observed. It is logical, however, to suppose that there is an inhibiting as well as a stimulating agent. Which predominates in the effect of an extract apparently depends on conditions at present unknown.

The stimulating substances contained in extracts do not seem to be class specific. Indeed, regeneration of frog liver and frog muscle is well stimulated with extracts of the corresponding tissues of rabbit and hen. Furthermore, the use of extracts of homologous frog tissues gives non-significant results. This surprising result might be considered doubtful were it not repeatable with such consistency. There is no doubt that it demands further study. At present one may assume that the stimulating activity of an extract mainly depends on the properties of the tissue donor and not on the degree of species proximity, and that extracts of tissues of higher vertebrates show a bigger stimulating activity than extracts of frog tissues.

The high specificity of action and the results of heat treatment of the extracts suggest that the stimulating influence is mediated by intracellular proteins. Saetren (1956) made such an assumption about the substances liberated from macerated organs which influence specifically the mitotic activity of the homologous organ. The assumption is confirmed by the experiments with radioactive methionine made by Ebert (1954) and Walter et al. (1956). It is possible, however, that a substance forming a complex with proteins and precipitating together with the protein on heating has the stimulating ability. Possibly RNA is such an agent. Ebert's data (1958a) are very interesting in this respect. He showed in experiments with chick embryos that the microsomal fraction of the homologous organ is the most active in selective accumulation. The same fraction gives rise to specific inductions (Yamada, 1958; Hayashi, 1958; Niu, 1958) though it is not determined whether the active agent here is the protein component or the RNA of a ribonucleoprotein complex. The participation of DNA or of desoxyribonucleoproteins is less probable. It is known that DNA shows a very low resistance to ionizing radiations (Tongur, Golubeva, Diskina, Spitskovski, & Filippova, 1957; Budilova & Kuzin, 1957), while the stimulating agent is rather radio-resistant (Tumanishvili, 1959a). It is clear that the stimulating factor of a tissue extract is connected with a water-soluble and thermo-labile protein fraction.

There are some reasons for assuming that the substances described form one of the means of physiological regulation of tissue formation during the process of regeneration. The liberation of these substances increases in the first, destructive, phase of regeneration; this causes the second phase of regeneration, the phase of reconstruction and differentiation, to begin and develop. Such an idea agrees with the fact that the second phase of regeneration proceeds the better the more intensive the first phase is (Polezhaev, 1947, 1956; Umanski & Kudokozev, 1951, 1952; Kudokozev, 1957; Rose, 1944).
SUMMARY

1. By means of systematic injections of tissue extracts one may obtain stimulation of regeneration of muscles (in frogs) and liver (in frogs, rabbits, guinea-pigs). Such extracts, however, apparently only influence homologous tissue.

2. The influence of extracts does not show any class specificity. On the contrary, the stimulation of regeneration of frog muscle and liver is more effectively produced by corresponding extracts of hen tissues than by those of the frog.

3. Aqueous extracts are as active as those obtained with isotonic saline.

4. Heating to 60° C. for 5 minutes inactivates the extract.

5. The analysis of our data and of those published elsewhere leads to the conclusion that the stimulating agent is a water-soluble protein fraction, or that it is some substance (e.g. RNA) forming a rather strong complex with this fraction.

RÉSUMÉ

Action stimulatrice des extraits de tissu sur les processus de régénération

1. Des injections systématiques d'extraits de tissus ont pour effet de stimuler la régénération des muscles (chez les grenouilles) et du foie (chez les grenouilles, les lapins, les cobayes). Ces extraits ne paraissent cependant avoir d'action que sur des tissus homologues.

2. L'action des extraits ne montre aucune spécificité de classe. Au contraire, la stimulation de la régénération du muscle et du foie de grenouille est plus efficace quand elle est provoquée par les extraits correspondants des tissus de poule que par les extraits de grenouille.

3. Les extraits aqueux sont aussi actifs que les extraits préparés avec une solution saline isotonique.

4. Le chauffage à 60° C. pendant 5 minutes inactive l'extrait.

5. L'analyse de ces faits et des données publiées ailleurs permet de conclure que l'agent stimulateur est une fraction protéique soluble dans l'eau, ou une substance (par exemple le RNA) formant avec cette fraction un complexe assez stable.

ACKNOWLEDGEMENTS

The author is grateful to Professor G. V. Lopashov (Institute of Animal Morphology of the Academy of Sciences of the U.S.S.R., Moscow) for his valuable advice, to Professor E. L. Andronikashvili (Director of the Physical Institute of the Georgian Academy of Sciences, Tbilissi) for the attention paid to this work, and also to Professor A. E. Lezhava and Miss E. Torotadze (University of Tbilissi).
REFERENCES


**EXPLANATION OF PLATES**

**PLATE I**

**Fig. A.** Frog muscle. The region of the lesion on the 20th post-operative day in a non-injected control. A 'funnel' which appeared as a result of the lesion is seen. The main part of it is unfilled; the rest is filled with fibrin and structureless material. Heidenhain's haematoxylin; oc. ×15, obj. ×6.

**Fig. B.** The same preparation as shown in fig. A, at the bottom of the 'funnel'. The ends of the cut fibres are seen. They are almost unchanged. Heidenhain's haematoxylin; oc. ×10, obj. ×40.

**Fig. C.** Frog muscle. The region of the lesion on the 20th post-operative day. Hen muscle-extract had been injected. The area formerly occupied by the 'funnel' is shown. Some muscle-fibres sprouting into the region of the lesion may be seen. Heidenhain's haematoxylin; oc. ×15, obj. ×6.

**Fig. D.** Detail from fig. C at higher magnification. Transversely striped newly formed fibres are seen. Heidenhain's haematoxylin; oc. ×10, obj. ×40.

**Fig. E.** Frog muscle. The region of the lesion on the 17th post-operative day. Hen muscle-extract had been injected. One can see a decaying fibre with nuclei coming out of it. Mallory; oc. ×15, obj. ×90.
Fig. F. Frog muscle. The region of the lesion on the 20th post-operative day. Hen muscle-extract had been injected. One can see longitudinally orientated myoblasts. Mallory; oc. ×15, obj. ×60.

Plate 2

Fig. G. Frog liver. The region of the lesion on the 12th post-operative day in a control specimen. The edges of the hole which formed as a result of the lesion are seen, so is part of a structureless mass filling it. The cells of the edge have also suffered destruction. Heidenhain’s haematoxylin; oc. ×15, obj. ×6.

Fig. H. The same preparation as shown in fig. G, showing the structureless mass filling the hole at a higher magnification. Heidenhain’s haematoxylin; oc. ×15, obj. ×40.

Fig. I. Frog liver. The place of the lesion on the 12th post-operative day. Hen liver-extract had been injected. Heidenhain’s haematoxylin; oc. ×15, obj. ×6.

Fig. J. Detail from fig. I at a higher magnification; oc. ×15, obj. ×20.

Fig. K. Frog liver. The place of the lesion on the 9th post-operative day. Hen liver-extract had been injected. An amitotically dividing nucleus is seen (indicated by an arrow). Heidenhain’s haematoxylin; oc. ×15, obj. ×60.

(Manuscript received 23:iii:59)