THE GROWTH AND DEATH OF TISSUE CULTURES EXPOSED TO SUPRANORMAL TEMPERATURES

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PLATE 29

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In an attempt to investigate the nature of the recovery of tissues from sublethal exposures to supranormal temperatures it was noted that cultures exposed to higher temperatures for short periods of time and then returned to an incubator at 39°C. would grow at essentially the same rate as controls kept continuously at 39°C. Exposures for longer intervals of time led to an inhibition of growth, and it was hoped that by employing various intervals of exposure at various temperatures some measure of this inhibition would be had. It is a matter of some interest also that beyond a certain critical exposure time *all* growth is inhibited and death ensues, and we have been interested to ascertain whether the lethal exposure represents a period in which all cells are killed directly or whether some factor necessary for their growth is destroyed. The departure of one of us from Germany before all the experiments could be completed prevented a complete answer to the problems we set ourselves. At the same time we believe that certain interesting implications in the available data are worthy of mention.

Method

The experimental material consisted of pure cultures of chick osteoblasts. The tissue was cultured ordinarily in Carrel flasks containing chicken blood plasma and a supernatant fluid consisting of 15 per cent embryonic juice in Tyrode solution. The supernatant fluid was changed every 3 days (see Fischer, 1930). Certain cultures were carried on in the ordinary way on cover-glasses, the medium consisting usually of equal parts of 25 per cent embryonic tissue juice and blood plasma. The ensuing account will be confined almost exclusively to the behavior of tissues grown in Carrel flasks.

The cultures were prepared by cutting the tissue of a healthy, growing culture of osteoblasts into two approximately equal sized pieces. One of the transplants, used as a control, was placed in a flask that was kept in an incubator at 39°C. The other transplant was exposed to the higher temperature by placing the flask in a water bath, and then returned to an incubator at 39°C. after a definite interval. Records of the growth of the cultures were made by daily measurements of their areas. Measurements were taken from 3 to 8 days after the initial transplantation. The inaccuracies ordinarily attributed to areal measurements are apparently of no great significance in our experiments, since both growth inhibition and death are very definitely indicated in the growth curves obtained.

Experimental cultures were exposed to five temperatures ordinarily considered supranormal, namely: 42°C., 44°C., 47°C., 50°C., and 52°C. In all the experiments to be described the temperature never varied by more than +0.1°C. during exposure periods, and usually the variation was much less. In order to insure rapid and effective thermal penetration the flasks were immersed in the water of the thermostat, except for certain experiments conducted at 42°C. where prolonged exposures necessitated placing the cultures in an air chamber that was in turn immersed in a properly heated water bath.

Our first, rather surprising, experience was that cultures growing at 42°C. would, apparently, continue to do so for an indefinite period. No lethal exposure period could be found. Furthermore, the growth of cultures at 42°C. continued at approximately the same rate as in the control cultures at 39°C. The only indications of any growth inhibition occurred in the so called "faster growing cultures" exposed for fairly long periods (Chart 1).

It will be seen that all cultures exposed to 42°C. for more than 30 hours continue to grow, but at a relatively slow rate, so that after 9 days of growth they attain an area of approximately 80 units. In fact the growth rates of cultures exposed to 42°C. for more than 30 hours are practically identical whether the exposure has been for 45 hours or 94½ hours, and no matter what rate of growth the control cultures exhibit. It is as though exposure to 42°C. beyond a certain time results in the induction of a definite slow growth rate. We have not exposed cultures to 42°C. for more than 130 hours and it is possible that extremely lengthy exposures might result in lethal effects.

Cultures exposed to 44°C. also proved exceptionally resistant. Certain cultures kept at this temperature for 124 hours continued to live and grow. In an attempt to discover if any lethal effect could be had a set of five cover-slip cultures was prepared. These were kept

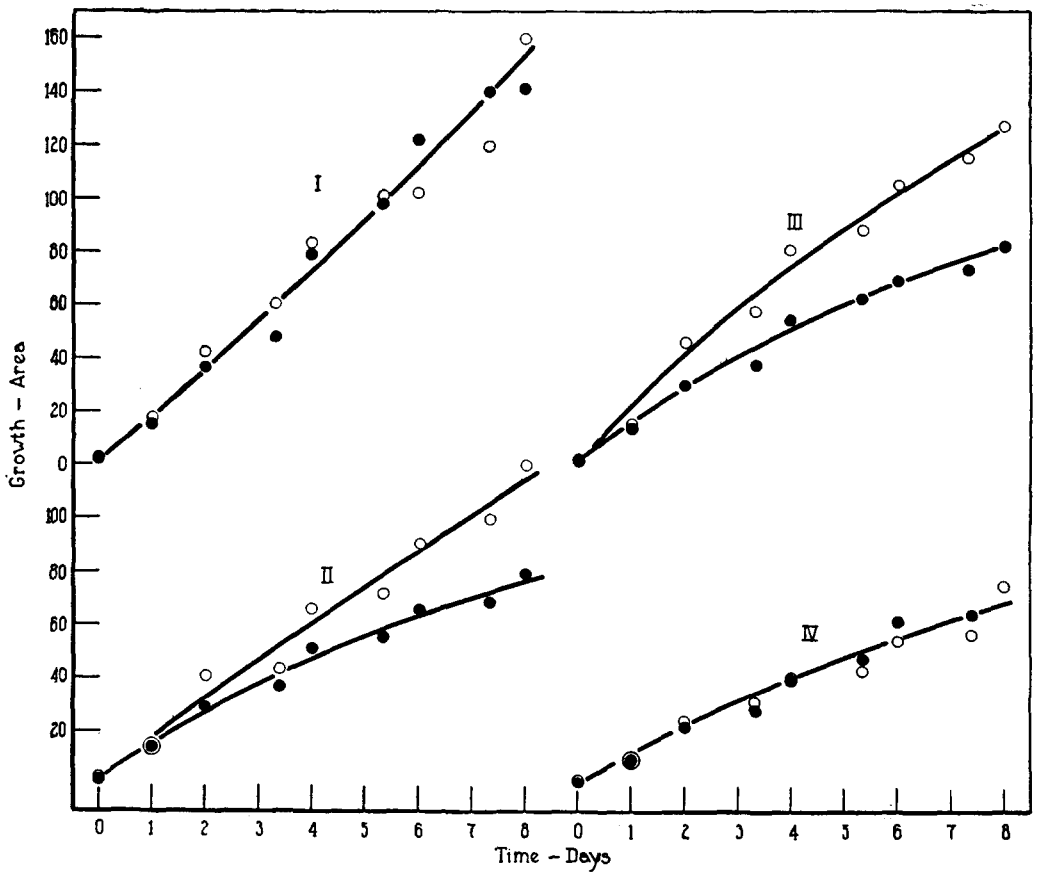

CHART 1. Growth of cultures in Carrel flasks exposed for varying intervals to 42°C.: I. Exposure ime = 30 hours; II. Exposure time = 45 hours; III. Exposure time = 54 hours; IV. Exposure time = 90 hours. In this and all succeeding figures open circles represent data for the control cultures at 9°C.; closed circles represent data for the experimental cultures. Note that for all exposures beyond 0 hours the experimental cultures grow at approximately the same rate regardless of the exposure ime or the growth rate of the control cultures.

at 44°C. and transplanted to fresh media every 2 days. After 144 hours one culture was still alive and four were dead. In another series one such culture showed the growth of a few cells after 168 hours exposure to 44°C.

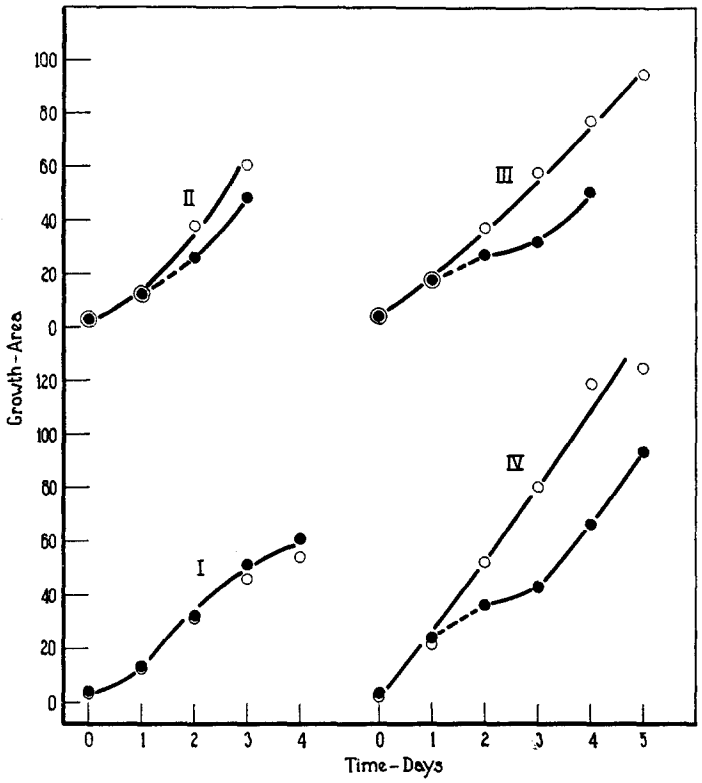

CHART 2. Growth of cultures exposed for varying intervals to 44°C.: I. Exposure time = 1½ hours; II. Exposure time = 3 hours; III. Exposure time = 15 hours; IV. Exposure time = 46 hours.

Despite the fact that no lethal exposure time was observed in our flask cultures, a very obvious inhibition of growth occurred in all exposures lasting 3 hours or more. This is graphically illustrated in Chart 2. The growth inhibition does not manifest itself until after an appreciable latent period; then, as the curves illustrate, the growth rate falls off even though the cultures have been returned to the incu-

bator at 39°C. before any effect on the growth rate is noticeable. This latent period preceding the change in growth, curiously enough, appears to be about 1 day in length whether exposures have been made for 3 hours or for 46 hours. Since our measurements were made every 24 hours it is difficult to decide exactly the length of this latent period, but it is certainly between 24 and 48 hours long in each case. We had

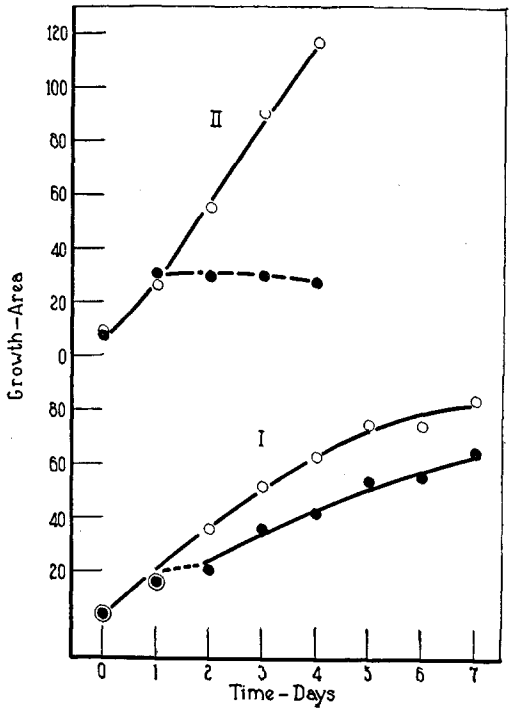

CHART 3. Growth of cultures exposed for varying intervals to 47°C.: I. Exposure time = 40 minutes; II. Exposure time = 60 minutes.

hoped to obtain indications of the reduction of this latent period with increasing periods of exposure, but no such indications are to be had, at least within the limits of determination possible with our methods of measurement.

One other consideration worthy of mention here is that cultures exposed to 44°C. for 3 hours or longer will show this definite inhibition of growth, although after a period of time the growth rate will again become almost normal.

When exposures were made at 47°C. definite lethal effects were observed. Cultures exposed to 47°C. for 1½ hours always die. Cultures exposed for 1 hour will in some instances die, in others live. Although cultures exposed for less than an hour invariably continue to grow there is a definite inhibition of growth in cultures exposed for

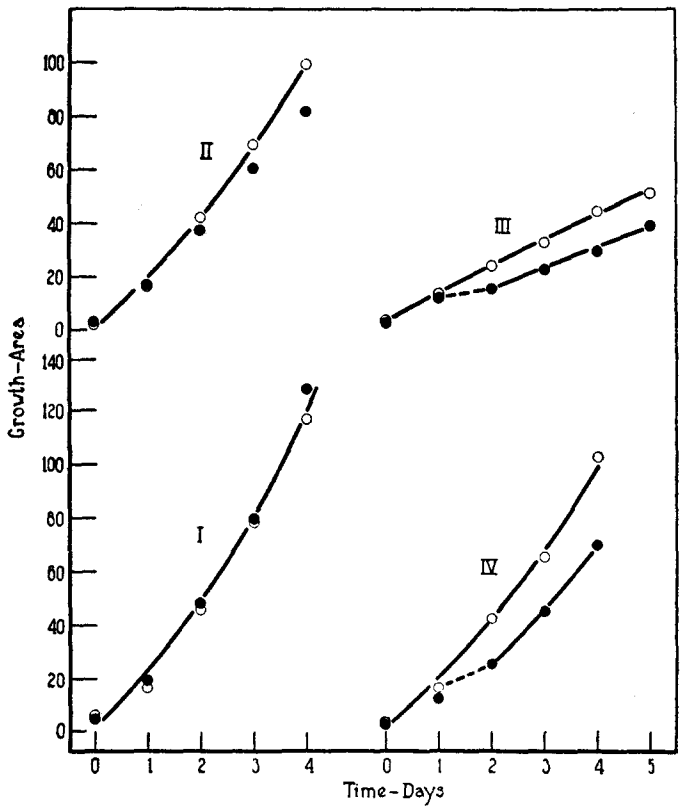

CHART 4. Growth of cultures exposed for varying intervals to 50°C.: I. Exposure time = 1 minute; II. Exposure time = 2 minutes; III. Exposure time = 3 minutes; IV. Exposure time = 4 minutes.

30 minutes. An exposure of less than 30 minutes results in no detectable inhibition of growth.

Here again the latent period preceding definite growth inhibition is approximately 24 hours, irrespective of the length of exposure. Similarly, in cultures which have been subjected to lethal exposures there

is a definite growth for 24 hours and then all growth ceases and death ensues. This is illustrated graphically in Chart 3 where the data are presented for a culture dying after 1 hour's exposure to 47°C. Note that apparently for 24 hours the experimental culture continued to grow at the same rate as its control, or at a slightly faster rate. Observe also that this 24 hour latent period preceding any manifestation of the effect of exposure is similarly exhibited in a culture receiving a sublethal exposure. In this and in all succeeding experiments we have

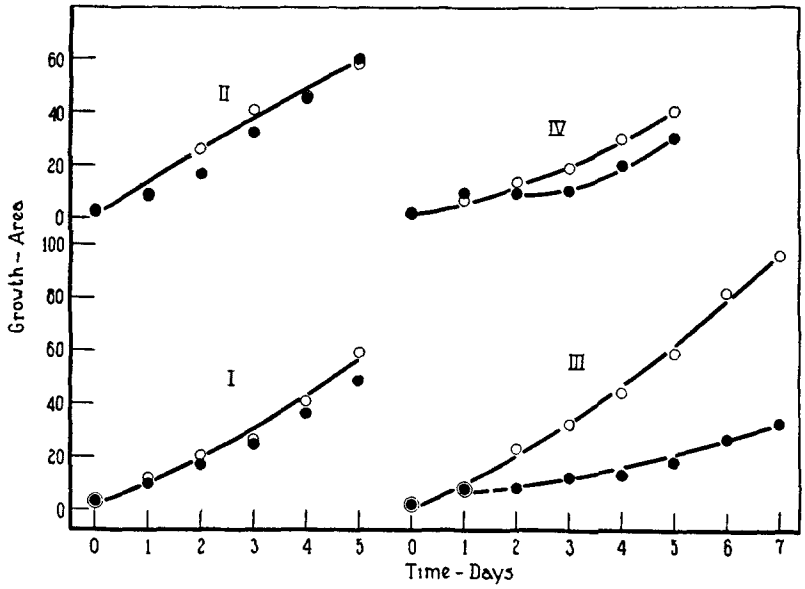

CHART 5. Growth of cultures exposed for varying intervals to 52°C.: I. Exposure time = ½ minute; II. Exposure time = 1 minute; III. Exposure time = 2 minutes; IV. Exposure time = 2½ minutes.

denominated an exposure as lethal when the culture exposed shows no growth after a 24 hour interval. We have verified the fact that cultures that cease to grow are really dead, by making transplantations of such cultures into fresh media. In no case was renewed growth observed, whereas all cultures showing continued growth after sublethal exposures did grow (and at a normal rate) on transplantation to fresh media.

The lethal exposure at 50°C. is between 5 and 6 minutes, whereas

growth inhibition is established definitely after 2 to 3 minutes of exposure. Again we notice that the manifestation of growth inhibition and death is not had until after 24 hours of growth (Chart 4). Our findings for exposures to 52°C. are similar, except that the lethal exposure is reduced to $3\frac{1}{2}$ minutes, the definitely inhibitory exposure to $\frac{3}{4}$ minute.

The findings for the five temperatures studied are summarized in Table I.

The data expressing the relation to temperature of the exposure time just necessary to induce growth inhibition can be fitted by the Arrhenius equation, and the value of the constant μ in this equation becomes 149,300 $\pm$ calories. Such a high value is characteristic of processes involving thermal destruction (*cf.* Brown and Crozier, 1927;

TABLE I

Temp.	Growth inhibition after exposure		Death after exposure
	Observed	Calculated (μ = 149,300)	
°C.	*min.*	*min.*	*min.*
42	?	—	?
44	180	215	?
47	30	24	105
50	2.5	2.7	6
52	0.75	0.65	3.5

Westermarck, 1927). The temperature coefficient for the actual death of the cultures in these experiments is also very high, but the data do not permit its accurate calculation. Most experiments on the "thermal death point" of tissues grown in culture are too scant to permit any reliable calculation of the temperature characteristic, though all indications are that it is very high. The chief concern of most experimenters has been to determine the exact lethal exposure time at temperatures just above normal. Friedgood (1928) for example, established that Walker rat sarcoma cells were killed by a 30 minute exposure to 44°C., but found no differences between the susceptibilities of the malignant cells of the tumor and the normal mononuclear elements. Other workers have also been concerned with the relative susceptibilities of malignant and normal tissues (for references see Friedgood, 1928; and Westermarck, 1927). We have been unable to discover, however, any detailed analysis of the growth of tissue

cultures in relation to temperature, particularly with reference to the inhibitory effect of sublethal exposures.

The only ascertainable morphological difference between cultures receiving sublethal and lethal exposures consists in a definite rounding up of the cells in the latter case. This is illustrated in Fig. 1 and is indicative of the death of the cells (*cf.* Kokott, 1930, *a*). Note the apparently flourishing condition of the culture receiving an exposure sufficient to induce marked growth inhibition (Fig. 2).

Lethal exposures, then, result in the death of the cells of the culture. But what factors govern the growth inhibition in sublethal exposures? We have ascertained that it is not a heat destruction of the growth factor in the embryonic tissue juice. Our experiment consisted simply of heating three samples of embryonic juice at 50°C. for 4 minutes, 5 minutes, and 7 minutes respectively. Tissue grown in media containing these samples as constituents showed no detectable alteration in its growth after 72 hours of growth, although an exposure of 7 minutes at 50°C. is sufficient to kill a culture and the shorter exposures induce marked growth inhibition.

It is possible that exposures to high temperatures caused the production of toxic substances in the blood plasma.[1] We have not tested this point, but are inclined to doubt the possibility. The experiments of Kokott (1930, *a*) indicate that exposures to supranormal temperatures result in a definite rounding up of the cells of a tissue culture. This rounding up is reversible with sublethal exposures but not with lethal exposures (see also Loeb and Drake, 1924). Exposures to high temperatures affect also the mitoses, reducing both the number of mitoses (Kokott, 1930, *b*) and the rate of mitotic division (Bucciante, 1927). There is then a direct effect upon the cells of the culture. The high temperature coefficient that we obtain for the growth inhibition indicates a "destructive" process. Whether this destructive process involves the death of increasing numbers of cells with increasing temperature, or the destruction of increasing amounts of a substance necessary for normal cell growth, we cannot decide. It is interesting in this connection to note that Mossa (1927), studying the growth of neurites at various temperatures, was able to discover only a few

[1] It has long been known, of course, that heating embryonic tissue juice for ½ hour at 56°C. destroys its growth-promoting power. Similar heating of the blood plasma at 56°C. makes it strongly growth-inhibiting.

slowly growing fibers at 46°C. At 44°C., however, he did apparently observe the usual number of growing elements, but these showed a reduced rate of growth compared with the maximum at 39°C. A reduction in the rate of biological processes beyond certain definite critical temperatures has, of course, been repeatedly observed (*cf.* Crozier, 1926; Crozier and Stier, 1927).

SUMMARY

In a study of the growth of chicken osteoblasts in cultures exposed to supranormal temperatures of 42°C., 44°C., 47°C., 50°C., and 52°C., it has been observed that:

1. There is no lethal effect after prolonged exposures at 42°C. and 44°C.

2. Cultures are killed after an exposure of 105 minutes at 47°C., of 6 minutes at 50°C., and of 3.5 minutes at 52°C.

3. A definite inhibition of growth occurs after different exposures at all temperatures from 44°C. onwards (see Table I).

4. There is a latent period of approximately 24 hours before any discernible effect of sublethal or just lethal exposures; this latent period appears to be independent of the duration of the sublethal exposure and of the temperature.

5. The high temperature coefficients for lethal exposures and for exposures just sufficient to inhibit growth indicate an underlying "destructive" process in the cells of the culture.

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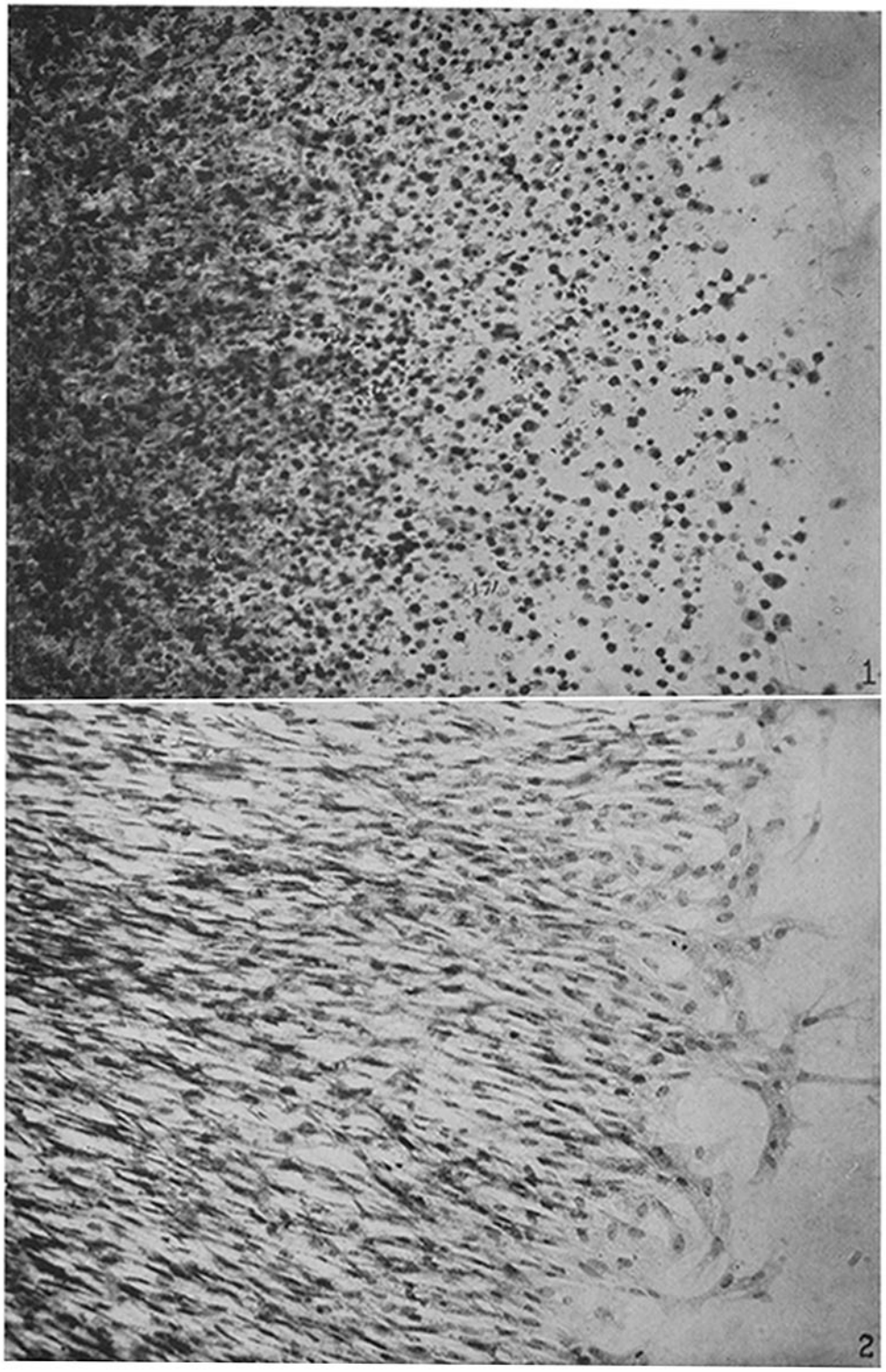
EXPLANATION OF PLATE 29

FIG. 1. Microphotograph of a culture exposed to 50°C. for 7 minutes. The staining was done with Haidenhain's iron-hematoxylin. × 165. See text.

FIG. 2. Microphotograph of a culture exposed to 50°C. for 4 minutes. The staining was done with Haidenhain's iron-hematoxylin. × 165. See text.



(Pincus and Fischer: Tissues under supranormal temperatures)