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HISTOLOGICAL CHANGES IN THE LIVER OF SALAMANDERS DURING STARVATION

Jean Stevenson

I.

The purpose of the present paper is to present briefly observations made on the microscopic structure of the liver of the salamander, *Ambystoma maculatum,* during inanition. As is well known (especially through the long-continued work of Professors C. M. Jackson and Sergius Morgulis) an organism, when prevented from obtaining its supply of energy through normal food-ingestion, can subsist for a time on its metaplasmic reserves. This condition of starvation, or "inanition", is accompanied by the many physiological, morphological, and chemical changes which always occur when the ordinary food-balance is upset and the individual is forced to use its own tissues as a source of energy-supply.

In the present short contribution I propose to consider changes observed in the liver-cells of Ambystoma, accompanying different degress of starvation: of 5, 10, 15 and 20 days, to be exact. Several factors will be considered: (1) the microscopic appearance of the cytoplasm—its consistency, granulation and vacuolization. Whatever conclusions can be reached concerning these properties will be made the basis of inferences in regard to the glycogen and fat-content after various periods of starvation. (2) The pigment-distribution, both in respect of its arrangement in the liver of each individual, and in respect of the amount present in each case. (3) There will also be considered the Kernplasma-ratio and its variations with the degree of inanition.

II.

Various investigators have studied the phenomena presented by the liver-tissue of starving amphibians. As will be seen in the admirable summary by Jackson (1929), and in Morgulis's collected work (1923), Trambusti forty years ago (1896) demonstrated an atrophy of the liver cells in *Spelerpes fusca* left two and one-half months without food. Sergius Morgulis also made (Morgulis, 1923) an extended study of inanition in the freshwater newt, *Diemyctylus viridescens.* He found a reduction in the cell-volumes in the liver with a relative increase in the size of the nucleus; he also observed granulation and vacuolization of the protoplasm and the accumulation of golden-yellow pigment granules both inside and outside the liver cells. Berg (1924) found a large accumulation of fat droplets in the liver-cells of Salamanders during winter fasting. Hartmann (1918) found in fasting tadpoles of the toad, *Bufo vulgaris,* an apparent increase in the size of the liver cells, while Hertwig (1920) saw a marked decrease in the liver, but did not mention the cell-size in fasting tadpoles of *Rana fusca.* Jordan (1929) studied the pigment distribution in the liver in *Triturus* and found wide variations between different individuals. He found, however, no consistent relationship between the amount of pigment present and the liver function. He cites Eberth (1867) as saying that the larger the amount of liver-pigment present, the smaller the amount of fat.

In other classes of vertebrates, also, similar studies have been made. Plehn (1914) noted that there was a progressive disappearance of fat from the liver cells of trout during fasting periods, but that the glycogen was never entirely exhausted; while Greene (1921) found the glycogen store of salmon much depleted during the spawning season. In starving eels, D'Ancona (1922, 1926, 1927b) found that the liver cells were reduced about ninety per cent in volume and the nuclei by a somewhat smaller amount; he also noted a congestion of blood in the liver, with an accompanying relative fibrosis. Esaki (1925) described atrophy of the hepatic cells and nuclear changes as taking place during starvation in the fish *Oryzias latipes;* and Smirnow (1913) studied the effect on rabbits of fasting with or without water, finding a greater fatty infiltration when water was not given. Several investigations have been made concerning changes in the relative weights of diverse organs during inanition in the mouse (Jackson, 1915; Stewart, 1918; Jackson & Stewart, 1919). Miller (1926) found a decrease in cell-size and number of cytoplasmic inclusions in the livers of starving rats; and Junkersdorff (1921) noted a persistence of fat and a greater relative loss of weight of the liver as compared with that of the body, in starving animals.

Adequate bibliographies of the subject of inanition have been presented by S. Morgulis (1923) and by C. M. Jackson (1929). The work of the latter, with its 727 summarized titles, has been of especial value in the writing of the foregoing summary.

III.

In the present study twelve individuals of *Ambystoma maculatum* were taken and killed under experimental conditions at intervals after periods of starvation of five, ten, fifteen and twenty days, respectively. There were thus, with controls, at least two specimens of each stage. The animals were kept under humid conditions, at a mean temperature of approximately 27° C. The liver, stomach and pancreas were removed and fixed in Zenker's fluid; and after the usual technique were cut into ten-micra sections and stained with hematoxylin-eosin.

Because several factors were to be considered, it was necessary to employ various methods for the study of the tissues. To ascertain the quantitative relation between the volume of the nucleus and of the total cell-mass, two methods were employed: (*a*) the micrometer measurement method, with subsequent calculation of volume of fifty representative cells, and (*b*) the weighing method in which fifty similar cells of each stage were projected upon thick blotting paper and drawn with the aid of a camera lucida. The nuclei were then cut out separately, and weighed; the whole cells were similarly cut out and weighed. By simple calculations the average cross-sectional areas of the nuclei were obtained, as well as the average cross-sectional areas of the total cells. The cells were assumed to be spherical, for simplicity's sake, and the volume calculated of cell and nucleus, for each stage. In this way I obtained ratios indicative of that which exists between nuclear- and cell-volume.

Two methods were also used in the study of the pigment distribution. (*a*) The first gave a quantitive relation between the pigmented and non-pigmented areas. The length of a section of liver was measured with a mechanical stage, and ten points were chosen along this line at each of which outline sketches of the pigment present in the field were made with the aid of the camera lucida. A circular piece of black paper having a small hole burned in the center was used as a stop in the eyepiece to reduce the size of the microscopic field. The sketches were transferred to thick white blotting-paper, and these cut out and weighed, and a percentage relationship established between the pigmented areas and the total visible field. (*b*) The second method was that of observation of the sections under a binocular microscope to ascertain the arrangement of the pigment in each section of the liver.

The appearance of the cytoplasm was observed under a 2 mm. oil-immersion objective, and counts were made of the number of granules present in the cells. Ten fields were chosen at random on five different slides of individuals of each period. The granules, easily distinguishable, were counted at a constant focal distance and their numbers recorded, thus giving comparisons of numbers present after diverse periods of starvation.

IV.

Pigment-distribution in the liver of Ambystoma. On study of the data, it was found that the amount of liver-pigment followed a general tendency to increase with starvation. Most of this was present in larger masses of brownish-yellow granules which usually were concentrated toward the center of the section, rather than at its periphery. By the technique already described the percentage of pigment in the total field, expressed as a volumetric relationship at representative points in the sections of the liver was obtained. To abbreviate my tables, the average percentage of pigment present in the different stages of starvation was found to be as follows: 5 days, 2.5%; 10 days, 3.7%; 15 days, 4.0%; 20 days, 5.3%. It will thus be seen that there was with my material a progessive increase with starvation in the amount of liver pigment.

The Cell-nuclear relation in the liver cells of starving Ambystoma. Two methods were employed, as indicated above. The results obtained by the micrometer measurement method shows that the nucleus/cell volume ratios increased a little more than 100% during a period of starvation of from five to twenty days. The data, extracted from my extended tables, when summarized give the following nucleus/cell ratios for varying periods of starvation: 5 days, 7.1%; 10 days, 11.3%; 15 days, 10.5%; 20 days, 14.9%. The weighing method gave results which did not show so great an increase in the nucleus/cell ratio as did the previous method, but the differences appear negligible. Two means of determining the nuclear volume were used, and the ratios for each value were calculated separately. The table below compares the results obtained by the two methods:

TABLE I. NUCLEUS/CELL RATIOS IN STARVING AMBYSTOMA

Period of starvation	Micrometer method	Weight method
5 days	8.7%	7.1%
10 days	12.0%	11.3%
15 days	12.3%	10.5%
20 days	13.2%	14.9%

Cytoplasmic Changes During Starvation. The cytoplasm appeared to be more coarsely granular in salamanders starved twenty days than in those killed at a shorter period. The total number and size of the vacuoles seemed to become less. Counts made of the number of granules under the immersion objective showed that although there was a wide range in values in each individual, *the average showed a decided decrease with increasing degrees of inanition.* This is clearly shown in Table II. The granules appeared to be progessively coarser and larger with increasing starvation.

TABLE II. NUMBER OF GRANULES PER FIELD

Period of Starvation	Lowest Number Found	Highest Number Found	Mean	Average
5 days	67	161	114	107
10 days	64	139	102	96
15 days	64	129	97	92
20 days	35	123	79	68

V.

The data presented by my experiments and observations indicate that there is a measurable increase in the pigment-content of the liver with progressive inanition. However, before any broad conclusions can be drawn, the experience of Jordan, who made an extensive study of urodele livers (1930) may be quoted:

> ". . . though variations in the amount of pigment in the livers of the experimental individuals might be correlated with the type or tenure of the particular experiment, as seems altogether likely, the specific significance of liver variation [in pigmentation] defies precise analysis, since similar variations are related also to different phases of presumably normal non-seasonal functional activity. . . ."

Individual variation, then, must be recognized as a factor in the interpretation of the final data, together with the experimental errors that always accompany work of this type. It must also be remembered that the numerical values have only a relative significance; but they are comparable since they were derived under similar conditions. An examination of the data reveals an increase of over one hundred per cent in the volume occupied by the pigment, in fifteen days, and the degree of change in the first and last five-day periods was approximately equal. It may be concluded, therefore, that pigment granules do accumulate with measurable regularity during starvation in *Ambystoma maculatum.*

As seen above, the comparative volumes of a cell and its nucleus constitute a relation known as the nuclear-plasmic ratio. Under normal conditions this ratio is fairly constant for each tissue in any given species. But it has been noted that during starvation this ratio increases, not because the nucleus enlarges but because the cytoplasm is greatly depleted without a similar change of volume occurring in the nuclei of the cells. Morgulis (1923) in a study made of starving fresh-water newts found, however, that during the first month of starvation the volume of the whole cell diminished 48%, and the volume of the nucleus 50%. During the second month of fasting, there was no further reduction in size of the nucleus, while the volume of the cell as a whole decreased still further until it was but 25% of its original volume. At the end of the first month of fasting, the nucleus occupied but one-twentieth the total volume of the cell; at the end of the second month, it was one-tenth the volume of the cell. So much for Morgulis's findings.

My own data show that the nuclear-plasm ratio was almost doubled in fifteen days (7.9% to 14.0%). The difference in the time required for these changes to occur in Morgulis's experimental animals as compared with my own may be due either to the fact that they were of a different species, or that they were kept at a different temperature. Since the metabolic rate of poikilothermous animals varies directly with the temperature, it is possible that the warm weather prevailing during my experiments induced more rapid changes in the cells. The divergences in the absolute values obtained for nuclei and cells by Morgulis and myself may also be explained by the fact that we studied different species.

The progressively coarser granulation described above was also noted by Morgulis (1923), but he gives no figures in respect of the degree to which it occurs. The decrease in number of granules per field may be due to a coagulation of the smaller granules into larger ones as a result of an imbalance of the colloidal constituents of the protoplasm during starvation. Probably the actual amount of cytoplasm also underwent reduction, but such a conclusion could not be justified from the evidence at hand,, because while the granules decreased in number, they also increased in size.

Studies made upon the glycogen and fat-content as measured by the presence of vacuoles appear to indicate that these substances underwent also a characteristic reduction during starvation.

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