THE IMPORTANCE OF DEXTROSE IN THE MEDIUM OF TISSUE CULTURES.

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Since the metabolism of sugar plays such an important part in sustaining animal life, it seemed of moment to ascertain what influence this substance might have upon cells of tissue cultures which, although living necessarily under more or less anomalous conditions, nevertheless furnish the most satisfactory method known at present for the study of the behavor of cells when exposed directly to any given substance.

Material and Method.

For this investigation over 500 cultures of the connective tissue of chick embryos (7 to 9 days incubation) were prepared in media from which dextrose had been omitted or which contained from 0.25 to 5 per cent of this substance. Various kinds of media were used: white of egg, amniotic or allantoic fluid from chick embryos after 12 or 14 days incubation, Locke's solution containing 10 to 40 per cent bouillon,[1] Locke's solution with from 0.1 to 1 per cent peptone, Locke's solution containing gelatin, Locke's solution with white of egg, and Locke's solution alone.

The cultures were neither bathed nor retransplanted, as the object was to note the effect produced by a given medium without renewing it.

To facilitate the recognition of any abnormal structures that might arise in the cells as a result of other factors in the medium, it was necessary to determine the structure of the normal connective tissue

[1] 85 cc. of NaCl 0.9 per cent plus KCl 0.042 per cent plus $CaCl_2$ 0.025 per cent plus $NaHCO_3$ 0.02 per cent plus 15 cc. of chicken bouillon plus dextrose 0.5 per cent is known as Locke-Lewis solution.

cell. For this, sections of chick embryos and films of fresh embryonic tissue were used.

Normal Cells.—The connective tissue cell of the young chick embryo was found to be large, irregular, and often spindle-shaped, with a large round or oval nucleus. In some cells the nucleus was bent, in others it was double. The cytoplasm was homogeneous except for numerous rod- and filament-shaped mitochondria, an occasional fat droplet, and in some instances a few minute neutral red bodies. The position of the centrosome was frequently indicated by a clear region near the nucleus. The processes were elongated and usually adherent to those of neighboring cells or to the cover-glass. The cells had the same characteristics in the sections and in the films, provided the films had been prepared in Locke-Lewis solution containing dextrose and were not kept under observation for more than a few minutes. These cells corresponded substantially to those described from sections of the embryo by Duesberg (1910), and in tissue cultures by Lewis and Lewis (1915), Levi (1916), and W. H. Lewis (1919). They did not contain either vacuoles or specific granules.

Abnormal Cells.—When these cells were placed in certain media, other structures made their appearance, some within a few minutes, others not for many days. These abnormalities were for the most part of two kinds: a clear, slightly refractive vacuole and a more or less opaque, rather large granule. In addition to these, there were sometimes giant centrospheres, blebs, liquefaction of the homogeneous cytoplasm, and an accumulation of fat in the cells.

The vacuoles were encountered much more frequently than were the granules. Although never seen in well fixed sections of embryonic tissue, they did form in the cells in film preparations, especially in normal salt solution or Locke's solution lacking dextrose. Prigosen (1921), who made a study of the connective tissue cells of the chick embryo in film preparations, not only describes the accumulation of vacuoles in these cells but points out that certain granules described by other investigators (Albrecht, 1903; Renaut, 1907) are probably also abnormal, in view of the fact that these observers employed the film method. In cultures, however, vacuoles seldom appear before the 2nd or 3rd day. In certain media their appearance was delayed

indefinitely, while in others the vacuoles accumulated in the cells until the latter took on a frothy or moth-eaten appearance as described by Maximow (1916) and W. H. Lewis (1919). It is difficult to determine the nature of the vacuoles as they occur under so many conditions; *i.e.*, starvation, degeneration, phagocytosis, lack of oxygen, influence of bacteria, and lack of dextrose. They formed in cells in what would seem to be a most nutritive medium, such as a plasma clot, and were lacking in cells in a medium composed only of salts and dextrose. They certainly seem to indicate the action of some deleterious influence, since, in almost every instance, the accumulation of vacuoles was followed shortly by the death of the cell.

The granules were present much less frequently than were the vacuoles. They never formed in cells in film preparations, but were often observed in cells in plasma cultures and were especially abundant in those grown in white of egg, as was shown in a previous communication (1921). They seemed to be material stored up by the cell attributable to the protein nature of the environment since they were observed only in media containing this substance.

Vacuoles may occur in cells which contain granules and, as previously shown, are especially prone to do so in cells in egg albumin. The granules and vacuoles found in plasma cultures have occasionally been mistaken for different phases of the same body (Maximow, 1916; Shipley, 1919), but this is undoubtedly not always the case, for, as has been shown in regard to cells in egg albumin (1921), the vacuoles may form in regions removed from that occupied by the granules and also, as will appear from these investigations, the formation of vacuoles may be prevented by the addition of dextrose to the medium.

Effects of Lack of Dextrose upon the Cells of Tissue Cultures.

The effect of the lack of dextrose upon the cells of tissue cultures was definite and pronounced, and inevitably resulted in the production of vacuoles. 212 cultures were prepared in various media lacking dextrose, and in every instance vacuoles were formed, after which the cells rapidly degenerated. On the other hand, media such as white of egg, amniotic fluid, and allantoic fluid, otherwise unsatisfactory for cultures, became favorable for the growths when a small amount of dextrose was added.

The lack of dextrose was especially detrimental to cultures grown in Locke's solution or in Locke's solution to which 15 per cent of chicken bouillon had been added. In these solutions without dextrose the cells became full of vacuoles and died within a few days, while in the same solutions containing dextrose they survived many days, depending upon the amount of this substance in the medium. Surprising to say, cells in solutions rich in protein but lacking in dextrose died much sooner than those in a simple salt solution containing dextrose.

Effects of Dextrose upon the Cells of Tissue Cultures.

Only cultures in Locke's solution containing 15 per cent chicken bouillon were used in these experiments, partly because the cells remain more normal in this solution and partly because of the difficulty of attempting to add definite percentages of dextrose to some of the other media.

When only 0.25 per cent dextrose had been added to the medium the cells grew luxuriantly but the formation of vacuoles was delayed for only a few days. The cultures were quite variable. In some the vacuoles developed after 3 or 4 days, while others remained normal for 6 or 8 days. All of the cultures (over 100) in this solution, however, did eventually exhibit vacuoles. These cultures seldom lived more than 10 days. While, as stated above, no attempt was made to add dextrose to the plasma medium, nevertheless it seems apt to mention in this connection that the vacuoles that appear in plasma cultures probably are attributable to the small amount of dextrose (supposedly about 0.25 per cent) which the plasma contains. Cultures that are frequently retransplanted into fresh plasma have a much larger amount of dextrose available and therefore remain normal for longer periods of time.

Cultures in media to which 0.5, 0.75, and 1 per cent dextrose had been added differed little in appearance. The growth in all these media was extensive, full of cell division, and survived from 2 to 4 weeks without forming vacuoles. In some of these preparations, after 2 weeks of healthy growth, the cells one after another rounded up and sank to the bottom of the hanging drop until, within the course of 3 weeks or a month, all the cells had degenerated. These

cultures died without forming vacuoles, the last cell being as normal in structure as those of the initial growth. In other preparations, after an interval of healthy growth, all of the cells rapidly formed vacuoles and all degenerated within the following day or two.

When larger quantities (2 to 5 per cent) of dextrose were added to the solution, the growth sometimes was not extensive, and in most instances it did not survive so long as that in the normal solution or in one containing 1 per cent dextrose, but these cells never contained vacuoles. The results obtained when such large quantities of dextrose were added were influenced by the change in hydrogen ion concentration of these cultures.[2] Cultures in normal Locke-Lewis solution, which has an initial hydrogen ion concentration of 6.8 or 7, had usually, upon degeneration, become pH 7.2 or 7.4. Those in media without dextrose had about the same final hydrogen ion concentration. On the other hand, the cultures in media containing as much as 5 per cent dextrose were distinctly acid (pH 5.6 to 6) when tested at degeneration, regardless of the initial hydrogen ion concentration of the medium. The rapidity with which death of the cultures took place seemed to depend upon the change in hydrogen ion concentration. The appearance of these cells after death was usually quite different from that of cells in media containing less dextrose. They did not round up or sink to the bottom of the drop, but remained spread out on the cover-slip, keeping their outlines as though coagulated into skeleton forms.

While the condition which arises in tissue cultures when the amount of dextrose in the medium is varied is somewhat analogous to that in diabetes, it is by no means due to the same cause. The acid which is found in cultures is probably due to the breaking down of dextrose, while the acidosis exhibited by the diabetic is presumably due to the partial combustion of fats resulting in the formation of fatty acids.

[2] The details of these results are given in another publication with Felton, who investigated the hydrogen ion concentration of tissue cultures in this laboratory, and for this reason will not be repeated here (Lewis, M. R., and Felton, L. D., The hydrogen-ion concentration of cultures of connective tissue from chick embryos, *Science*, 1921, liv, 636).

SUMMARY.

It is not advisable to enter into a discussion of these findings at the present time, owing to the lack of experimental evidence as to the exact nature of the vacuoles. Regardless of the nature of these bodies or of what factors produce them, they are structures abnormal to the connective tissue cells. The lack of dextrose in the medium of tissue cultures leads to some condition distinctly detrimental to the cells, resulting in their vacuolation and death, even when the medium contains abundant protein material. The addition of small amounts (0.5 to 1 per cent) of dextrose to the medium delays the formation of vacuoles and prolongs the life of the culture. The addition of large amounts (2 to 5 per cent) prevents vacuolation of the cells, but so much dextrose usually leads to a change in the hydrogen ion concentration of the culture resulting in an acid condition which arises coincidentally with the degeneration of the cells.

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