SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

One hundred thirteenth meeting.

College of the City of New York, New York City, February 16, 1921, President Calkins in the chair.

62 (1644)

The reserve energy of actively growing embryonic tissues.

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In 1902 Fletcher[1] showed that the sartorius muscle of a frog will give a maximum contraction when stimulated every five minutes over a period of two hours in an atmosphere of pure nitrogen. In an atmosphere of oxygen, the same muscle will contract for a much longer time and the fatigue developing in an atmosphere of nitrogen may be removed by placing the muscle again in an atmosphere of oxygen.

If differentiated muscle has sufficient reserve energy to allow it to contract for a period without oxygen, the question arises, will not the same reserve be found in an actively growing tissue.

In a previous article in 1917[2] the author described a method which allows one to study quantitatively the effect of various concentrations of oxygen on the growth of cells *in vitro*. In that paper the main point studied was the relative effect of pure oxygen and various dilutions of oxygen in nitrogen on the rate and extent of the growth. The tissues used were fragments of heart muscle and other tissue of chick-embryo of various ages. These experiments showed that the growth was practically the

[1] *J. of Phys.*, 1902, xxviii, 474.

[2] *Am. Jour. of Phys.*, 1917, xliii, 13.

same in an atmosphere containing 8 per cent. of oxygen as in one containing a much larger proportion of this gas or in a pure oxygen atmosphere.

A low concentration of oxygen beyond which no growth of cells will take place was also recorded. But as stated this was not accurately determined. There were certain discrepencies in the method of measurements and as must be stated here the tissues used for determining this point were fragments of the heart of 15, 16, and 18 day old chick-embryos.

In the present experiments, these objections to the technic used as outlined in the previous paper have been removed and tissues from embryos of various ages have been tested. The tissues chiefly used have been fragments of the heart muscle and body wall of 4 and 5 day old, 10 day old, and 15 day old chick-embryos.

One criticism of Fletcher's work is that he used a muscle of considerable thickness and in which the gaseous exchange must have been slow. I think, however, as Bayliss states, that this objection was obviated by his second experiment where he placed the fatigued muscle from the atmosphere of nitrogen into one of oxygen and noted a recovery from the fatigue.

I measured the distance oxygen diffuses readily into clots of blood, clots of plasma, and into tissue by means of the color changes in red cells. Into clots of chicken's blood the diffusion takes place readily only into a surface layer of 0.5 to 0.7 mm. in thickness. The hemoglobin in red cells lying below this layer or which are separated from the air by a layer of clotted plasma of that thickness becomes readily and permanently reduced. In this surface layer they maintain a bright red color.

In tissues the diffusion of oxygen may be more. It may exceed 3 mm. There are variations, however.

In all of these experiments fragments of tissue less than a mm. in diameter were used. They were planted in a layer of plasma 0.5 mm. or less in thickness. The culture chambers were made from one piece of glass tubing prepared according to the manner described in the previous papers (loc. cit.). The gas to be tested was passed through the chamber intermittently for from 1 to 2 hours. The capillary tube inlet and outlet were then sealed in the flame.

About the fragments of 4- and 5-day-old chick embryos in an atmosphere of pure nitrogen, the heart muscle cells were seen to grow a short distance out into the medium, and there was also an active growth and division of the serosa cells. A similar growth of cells was also seen about the fragments of the body wall. Dividing figures were seen in both types of cultures.

This growth commenced generally, however, only after a considerable latent period varying from 10 to 24 hours. It continued actively for only a few hours, when it ceased and an active rounding of the cell and fragmentation of the nucleus and the cytoplasm intervened.

In 7.48 per cent. oxygen or in air, this growth commenced always much earlier even after one or two hours. It continued actively for from 24 to 72 hours, when it slowly ceased. The cells did not disintegrate at once, but very slowly. Even after weeks or months, many have been found in a good state of preservation.

No growth was seen in the cultures of 10- or 15-day-old embryos in an atmosphere of pure nitrogen. A growth comparable to that of the younger embryos in pure nitrogen occurred about the fragments of 10-day chick embryos in an atmosphere containing 1.8 per cent. oxygen, and about the fragments of the 15-day-old chick embryos in an atmosphere of 5.4 per cent oxygen in nitrogen.

The results of these observations are graphically shown in the accompanying table and curve.

Heart muscle fragments of young chick embryos or the whole hearts will contract at once, when placed in the plasma of a tissue culture and warmed to the temperature peculiar to them. Fragments of older chick embryos and fetal chickens will not contract in the culture until a certain amount of growth and movement has taken place.

Fragments of the heart of 4- and 5-day-old chick embryos were found to contract actively in an atmosphere of pure nitrogen for as long as 5 or 6 hours. After this time the contractions became generally weaker, but often did not cease entirely for from 20 to 24 hours.

CONCLUSIONS.

1. These experiments with the contracting heart muscle fragments are in complete harmony with Fletcher's much earlier ones.

2. The cells of young embryonic tissue contain also a reserve energy. They may grow in an atmosphere of pure nitrogen for a short time. This absence of oxygen leads soon, however, to their rapid disintegration. Oxygen is evidently necessary for their preservation as Warburg conceived it.[1]

3. This ability for the cells to grow without oxygen is lost with development. It fails in the case of the cells of older embryos;

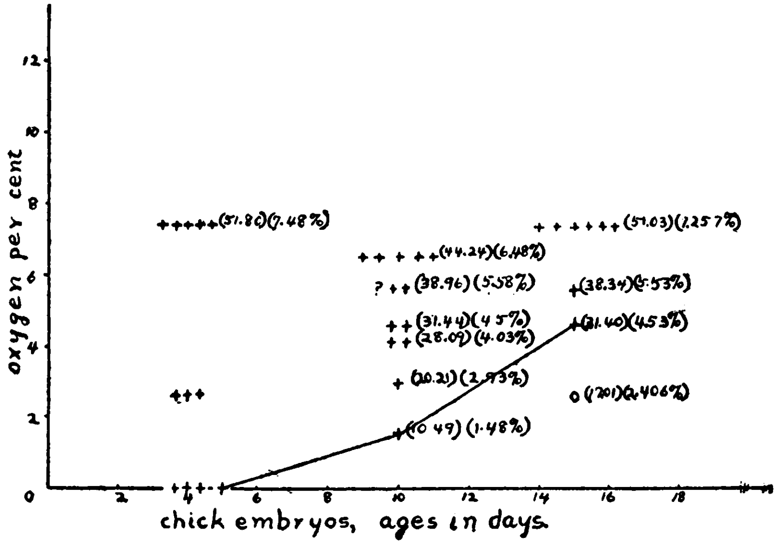

TABLE AND CURVE 1. The Relative amount of growth in the cultures is indicated by the number of + signs. That noted in the 4 day old embryonic tissue in 7.48 per cent. oxygen is comparable to what is seen in air.

as development proceeds, a greater and greater amount of oxygen is needed.

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Does growth require preformed carbohydrate in the diet?

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Carbohydrates are ordinarily regarded as indispensable components of the food intake. This belief is based on the presence

[1] *Ergeb. Phys.*, 1916, xiv.