3208

Disintegrative Action of KNC on Amblystoma Embryos in Solutions of Different Osmotic Pressures.

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A series of experiments were performed dealing with the effect of varying the osmotic pressure of the solution, on the disintegrative action of potassium cyanide, on the early embryos of *Amblystoma punctatum.* The experiment was carried on during the Spring of 1926. Owing to the unusually short season, the work is incomplete in many respects, and further experiments are planned for future seasons. However, certain very definite results have been obtained, which appear to have an important bearing on the general problem of susceptibility and susceptibility gradients.

In each experiment a batch of embryos from a single female was selected and dissected free from the jelly capsules. This was necessary because preliminary experiments showed that the capsules afford some protection against KNC. The embryos were allowed to remain for a number of hours in cold running tap water, during which time any that had been injured died. To freshly made solutions of commercial cane sugar, Ringer's solution, urea (made up in both distilled and tap water), and to distilled and tap water was added sufficient KNC to make the desired concentration. The range of concentrations employed was: Sugar, 0.03 molal to 0.6 molal; Ringer's solution, 1/10th to double the usual concentration; urea, 0.03 molal to 0.6 molal; KNC M/250 to M/125. As regards the osmotic pressure of the solutions, a crude cyroscopic method served to demonstrate that solutions of sugar of the order of 0.15 molal and Ringer's solution diluted one-half are both hypertonic for the Amblystoma embryo in the stages employed. Saturation with oxygen was assured by shaking the solutions with air. The embryos were then placed in 250 cc. Stender dishes in tap water, and the water replaced by 250 cc. of the desired solutions. The lids of the dishes were sealed with vaseline. Examination of the condition of the embryos was made at suitable intervals, and a record was made of all that showed any indications of death or disintegra-

tion. Depending on the number of embryos in the batch, groups of ten or twenty were placed in each dish. The stages used were blastulae and gastrulae. No attempt was made to bring the solutions to neutrality, hence, the results are the sum of the specific effect of the reagents and that of the alkalinity of the solutions. The distilled water used was once distilled in a copper still equipped with a block tin condenser.

From the data obtained in 15 experiments thus far completed, the following facts are clear: (1) Embryos die more rapidly in distilled water solutions of KNC than in like concentrations made up in tap water. The difference is equally evident between distilled and tap water solutions of urea, hypertonic sugar, and hypertonic Ringer's, to which KNC has been added. The additive action of KNC and distilled water of the sort employed here is, therefore, plainly not due to the hypotonicity of the KNC-distilled water solutions.

(2) In general, embryos disintegrate more rapidly in solutions of cyanide made up in Ringer's solution in at least two concentrations (full strength and one-half strength) than in such solutions made up in tap water. This additive action of KNC and Ringer's solution makes it necessary to observe caution in drawing conclusions regarding the rôle of osmotic pressure in the disintegrative action of KNC from results obtained with Ringer's-cyanide solutions.

(3) Urea is toxic in solutions iso-molal with non-toxic hypertonic concentrations of sugar. Conclusions regarding the effect of hypertonic solutions on development as compared with the effect of iso-molal solutions of urea are, therefore, of limited value.

(4) Cane sugar exerts an antagonism toward the disintegrative action of KNC. The protection afforded by the sugar increases with increasing concentration of the sugar up to 0.3 molal. There were no exceptions observed. The results indicate that with increasing concentration of the cyanide, the concentration of sugar necessary to afford greatest protection increases.

Embryos which were intact in strong sugar-cyanide solutions at the end of 48 and 72 hours were transferred to the same concentration of sugar without the cyanide, and the concentration of sugar decreased gradually by the addition of tap water. Some of the embryos disintegrated very soon, others developed slowly

for three or four days and then died. From this it may be concluded that while sugar protects the embryo against the disintegrative action of the cyanide, nevertheless the injury is profound and complete recovery does not occur after the exposures employed here.

Indications of death of the embryos differ among the solutions used. In one-half Ringer's solution plus M/250 KNC death in blastulae begins as a gray cap at the animal pole. This cap increases in area and spreads downward and eventually involves the entire surface. Blastulae do not usually burst during the first 72 hours of exposure. The process is similar but much more rapid in 0.15 molal urea plus KNC, and in tap and distilled water solutions of KNC, and is frequently accompanied by the bursting of the blastulae in the vegetal hemisphere. In the case of gastrulae, the region of the closing blastopore is first affected in these solutions. Visible changes are the whitening of the cells about the blastopore, particularly the dorsal lip, followed by the spreading of the whitened area anteriorly involving the neural plate. The neural plate may persist for some time as an area of dead tissue before other regions of the embryo are visibly affected.

Deaths occurring in hypertonic sugar-KNC solutions do not show this differential susceptibility of the animal pole, blastoporic lips, and neural plate. As stated above, death and disintegration in hypertonic sugar-KNC solutions is greatly delayed as compared with the rapidity of these processes in KNC added to the other agents employed. When disintegration finally does occur, no differences in the appearance of the animal pole of blastulae, nor the lips of the blastopore and neural plate of gastrulae, and other regions of the embryo are discernible. There were no exceptions to this among the more than 50 embryos dying in sugar-KNC solutions that were observed. The entire embryo becomes a lighter color and eventually bursts in the region of the yolk cells. In 0.6 molal sugar plus KNC whitened ridges appear, probably due to shrinkage in this concentration of sugar. The processes of disintegration begin at these ridges and soon spread and involve the entire embryo.

Two main conclusions may be drawn from the results of the experiments with sugar-cyanide solutions; first, as regards the disintegration of Amblystoma embryos, KNC and hypertonic sugar solutions are antagonistic. This fact is in line with the well

known experiments of Loeb, who employed KNC to prevent the disintegrative action of hypertonic sea water on sea urchin eggs. Second, hypertonic sugar solutions obliterate the differential susceptibility of the several regions of the embryo to cyanide disintegration. Observations on a possible similar action of strongly hypertonic Ringer's solution are incomplete.

3209

Effect on Blood Sugar of Direct Irradiation of Blood in Vivo.*

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In a paper previously published[1] one of us described a method of irradiating the blood of etherized dogs by allowing a beam from a special carbon arc to fall on a quartz tube inserted in the carotid artery. By this method a series of studies has been undertaken on the effects of this procedure on blood pressure,[1] on leucocytes,[2] on electrophoretic potential of erythrocytes,[3] on blood uric acid,[4] blood calcium,[5] and CO_2 combining power.[6]

It is the purpose of this paper to report the results of this procedure on blood sugar, for the sake of a more complete picture of the effects of such a method of irradiation.

A control series was first run in which the dog was subject to ether anesthesia together with all the operative technic but without irradiation. The first sample of blood was drawn from the saphenous vein before anesthesia. Subsequent samples were drawn from the femoral vein at various intervals. All blood samples were heparinized either *in vitro* or *in vivo*. Determinations were made by the method of Tolin and Wu.[7] The results of the control experiments are shown in Table I.

Another series was made in which irradiation was begun after

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