by this means they could be more rapidly built up and maintained. In most cases these transfers have been very successful, only a few of the colonies have succumbed. The failures may have been due to the normal hazards accompanying transfers, but the possibility is recognized that a species complex may exist in the potato populations. It is possible that intimate relationships between plant-parasitic nematodes and fungi may prove to be more common and important than has been hitherto suspected.

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Manuscript received September 11, 1953.

The Separation of Neuroblasts and Other Cells from Grasshopper Embryos[1]

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In the course of our studies of the effects of x-rays on the metabolism of grasshopper embryos (*Chortophaga viridifasciata* DeGeer), it was desirable to have suspensions of free cells in as near normal morphological and physiological condition as possible. Adequate suspensions have been obtained through enzymatic digestion of the intercellular substance. Neuroblasts as well as other types of embryonic cells from these suspensions undergo mitosis and maintain active respiration for several hours after separation. The method developed, somewhat similar to the method of separation of chick embryo cell suspensions described by Moscona (*1*), utilizes a digestive mixture of trypsin and hyaluronidase in a calcium-free medium at pH 7.4. Trypsin was essential for adequate solution of the intercellular substance, whereas the development of gel in the medium was lessened considerably by the addition of hyaluronidase. It is well known that calcium-free media decrease the adhesiveness of embryonic cells (*2, 3*) and cells of mature invertebrate and vertebrate tissues (*4–7*).

Routinely, 150 embryos (average total dry weight, 5.25 mg), selected morphologically at a stage equivalent to that of 14 days development at 26° C, were removed from the eggs under aseptic conditions in an isotonic salt medium containing calcium. After rinsing twice in a calcium-free medium to remove residual calcium, yolk, and egg fluid, the embryos are placed in 5 ml of digestive mixture for 1 hr at 38° C. The digestion mixture consisted of 7.5 mg each of recrystallized trypsin (Worthington) and bovine testis hyaluronidase (Armour or Mann) in 10 ml of calcium-free solution. Agitation during the period of digestion was avoided as this resulted in very viscous solutions, or gel formation, and clumping of the embryos. At the end of the hour 0.5 ml of protein solution (5 mg of Armour bovine albumin/0.5 ml calcium-free medium) was added to the digest suspension. Holter and Zeuthen (*8*) used egg white to decrease the adhesiveness of ascidian eggs to glass or to one another, and in our experience the albumin aided in overcoming stickiness among separated cells. At this stage the general form of the embryos was still distinguishable. Individual cells were separated by forcing embryos and solution slowly into and out of a small pipet with a rather large bore. Separation must be done carefully and gently for mechanical damage results in "bleb" formation at the cell surface, and few or no dividing cells can subsequently be observed in the suspension. When the solution is quite milky and all large particles have been broken up, it is transferred to a centrifuge tube with 5 ml of culture-medium containing calcium. After centrifugation at 158 rpm on a clinical centrifuge for 15 min (distance from axis to bottom of tube, 21 cm), the residue was resuspended in 0.6 ml of fresh medium. Cell separation was fairly complete, and no evidence of reaggregation of separated cells has been obtained. Hemocytometer counts of a typical suspension gave 2,000,000 cells, or about 13,300 cells/embryo, a figure which is undoubtedly low because of the unavoidable loss of cells during separation.

In Fig. 1 is shown the endogenous oxygen consumption of a cell suspension containing 1,732,000 cells measured at 38° C, in an atmosphere of oxygen. Respiration decreases after 3 hr, possibly due to lack of substrate. The average rate of oxygen consumption of the suspension shown in Fig. 1 was 12.7 mm^3 of oxygen/hr at 38° C. In Warburg determinations of the respiration of intact embryos, we have obtained an average value of 31.5 mm^3 of oxygen/hr for 150 embryos. The difference between these two values, 31.5 and 12.7, may be a measure of the yield of embryonic cells in the suspension, but with the evidence at hand

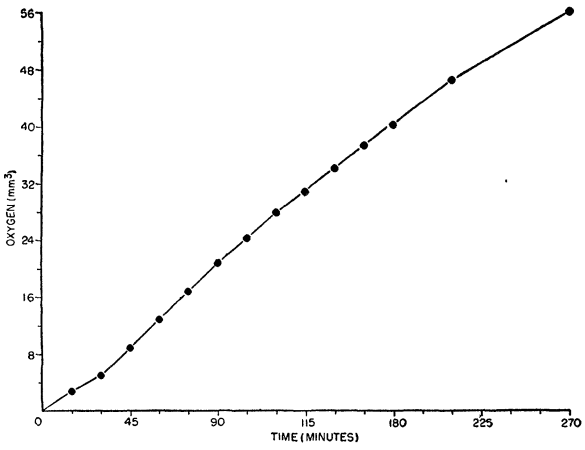

FIG. 1. The endogenous respiration of a grasshopper embryonic cell suspension in isotonic salt solution.

[1] Work performed under contract No. W-7405-eng-26 for the Atomic Energy Commission.

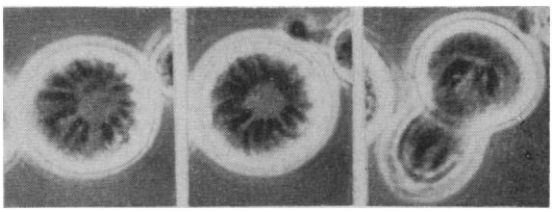

FIG. 2. Three views of a neuroblast cell in anaphase in a typical embryonic cell suspension prepared from a trypsin-hyaluronidase digest in calcium-free salt solution. Rotation of the cell has occurred so that two of the photomicrographs are polar views and the third is a side view. Neuroblasts normally divide unequally: the smaller daughter cell is the ganglion cell. Other cell types in the suspension divide equally.

it is not possible to determine whether the low value of cell respiration in the suspension is due to loss of cells in the separation, or to a decreased rate of respiration per cell.

For 7–8 hr after separation, dividing cells can be seen in suspension (Fig. 2). Detailed observations on division have been limited to mid-mitotic phases, and these phases proceed as in the intact embryo, but cursory examination of other phases has revealed no deviation from the normal. Second divisions of neuroblasts in the suspension have been observed.

It should be feasible to isolate neuroblasts or other types of embryonic cells from such a suspension for particular cytological or physiological study. Although the use of this technique has been limited to grasshopper embryos, it should be adaptable to other embryonic as well as adult tissues where a cell suspension or isolation is desirable.

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Manuscript received August 21, 1953.

Factors Influencing the Uptake of Iron by Blood and by Bone Marrow Cells *in Vitro*

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To investigate the mode of action of copper in iron metabolism, the technique used by Walsh *et al.* (*1*) to determine iron uptake by cells was used. The iron used was a mixture of Fe^{55} and Fe^{59} [2] and had a specific activity of approximately 2 mc/g Fe. It was added to the culture medium as ferrous ammonium sulfate. The blood cells were obtained from rabbits that were bled repeatedly to increase the reticulocyte count. The bone marrow cells were obtained from chicks or turkey poults.

The amount of iron uptake is dependent upon the washing of the cells in 0.93% NaCl prior to incubation. One milliliter of heparinized blood (reticulocyte count 6%, hemoglobin 9.5%) gave a count of 150 cpm after 8 hr of incubation. When washed once in 3 volumes of saline, before incubation, a count of 640 cpm was obtained. The 100 μg of Fe used gave a count of 25,700 cpm. In another experiment (11.7% reticulocytes) the cells were washed one, two, and three times and counts of 540, 900, and 930 cpm, respectively, were found. The influence of copper, the number of cells, and the iron level are shown by the data in Table 1.

TABLE 1.

Number of cells (washed once)	100 μg Fe 9 μg Cu (cpm)	100 μg Fe (cpm)	50 μg Fe (cpm)
1.63×10^9	1770	460	440
3.26×10^9	950	650	460
6.52×10^9	880	620	610

The addition of copper increases the iron uptake, particularly with fewer cells. Reducing the amount of iron has little effect. Where copper is present, fewer cells take up more iron than do larger numbers. In another trial, halving the iron approximately halved the uptake.

Similar results were found with bone marrow cells. These were obtained by crushing the long bones in isotonic saline, filtering through glass wool to remove extraneous material, and further isolation by slow centrifugation, following the technique of Magnussen (*2*). When resuspended in saline and again centrifuged, the washed cells took up 2.4 times more iron than those not washed. A second washing caused no further uptake. The effect of iron and copper levels is shown in Table 2.

It can be seen that as the level of iron supply is increased, the amount of iron uptake is increased. In-

TABLE 2.

μg Fe added	μg Cu added as the acetate (cpm)			
	0	5.7	11.4	22.8
10	270	350	440	870
40	1300	1800	1800	3000
120	4700	7400	7400	8300
120	3600*			200*

* Incubated 20 min; others, 6 hr. Total cells/culture tube 1.25×10^8: 100 μg Fe gives 12,700 cpm. Each datum is the average of 2 runs. Cells were from 5- to 6-wk-old chicks.

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