Technical Papers

The Mass Isolation of Whole Cells from Rat Liver[1]

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In a study of the effects of various perfusion media on cell breakage during homogenization, it was noted that the use of mixtures of equal parts of isotonic sodium citrate and calcium-free Locke's solution resulted in a suspension consisting mostly of whole cells (*1*). The effects of the inclusion of several divalent-ion binding substances in perfusion media on the percentage of whole cells in homogenates prepared under standard conditions will be considered here, and a method for preparing whole cells in about 40–50% yield will be presented.

Previously, liver whole cells have been prepared in low yield (5–10%) by forcing liver through cheesecloth (*2*), by shaking slices with glass beads (*3, 4*), by mincing with a tissue press (*5*), or by homogenization with a Potter-Elvehjem grinder (*6*). Purification has usually been achieved by centrifugation. The use of liver whole cell preparations, as pointed out by Potter (*7*), appears to offer promise (*4, 7, 8*).

The intercellular cement is generally considered to consist, for the most part, of a slightly soluble calcium salt. Removal of the calcium by the use of calcium-binding substances, such as citrate or oxalate, results in a softening of this cement (*9–12*). In the experiments reported here pyrophosphate, ethylenediamine tetraacetate (Versene), glycerophosphate, and adenosinetriphosphate (ATP) have been studied in addition to citrate. Versene is well known as a chelating agent, and ATP has been shown by Neuberg and coworkers (*13*) to complex a number of divalent cations.

Adult rats of the Sprague-Dawley strain were stunned, decapitated, and perfused through the dorsal aorta until the livers were well blanched with the solution under investigation. In the majority of the experiments back perfusion via the hepatic vein was also employed. Perfusion was generally completed within 3–4 min after the rat was stunned. The livers were excised and weighed in a tared beaker of cold calcium-free Locke's solution. Sufficient solution was removed to give a ratio of 1 g of liver to 10 ml of final suspension. The liver and solution were then transferred to a Pyrex homogenizer tube, and the liver broken up by pressing down with a loose-fitting lucite pestle. This was followed by twenty even up-and-down strokes by hand. Shreds of connective tissue containing many cells remained after this treatment, but they were readily removed by straining through 10XX bolting silk. Experience has shown that further homogenization to release more whole cells increased the contamination with fibrous debris and broken cells and did not give a much greater yield, owing, evidently, to breakage of cells previously released. Cutting the liver up into small cubes or slices with a razor did not appear to improve the yield.

Perfusion solutions were made by mixing approximately isotonic solutions of the sodium salt of the complexing agent with calcium-free Locke's solution in ratios ranging from 1 : 3 to 1 : 19. The final solutions were adjusted to pH 7.0 with HCl or NaOH as necessary.

The relative numbers of nuclei and whole cells were counted in small aliquots, dark-medium phase contrast being used. A total of approximately 1000 whole cells and nuclei were counted in each preparation. Since approximately 31% of the liver cells of the adult rat are binucleate (*3*), the number of cells broken may be calculated by dividing the number of nuclei by 1.31. This value, added to the number of whole cells counted, gives the number of cells contributing to the samples counted. The percentage of whole cells was calculated by dividing the number of whole cells counted by the

TABLE 1

EFFECT OF INCLUSION OF VARIOUS SUBSTANCES IN PERFUSION MEDIUM ON PERCENTAGE OF WHOLE CELLS IN HOMOGENATES

Substance	Molarity	% whole cells
Citrate	0.027	38*
	0.027	31*
	0.027	69
	0.027	47
	0.0138	49
	0.0138	56
Pyrophosphate	0.030	60
	0.030	73
	0.015	56
	0.006	12
	0.006	14
Versene	0.0276	60
	0.0138	67
	0.0138	49
	0.0055	57
	0.0055	55
Adenosinetriphosphate	0.0138	64
	0.0055	52
	0.0055	31
Glycerophosphate	0.035	16
	0.0175	17
Complete Locke's	—	6
	—	12
Ca-free Locke's	—	14
	—	12

* Back perfusion omitted.

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

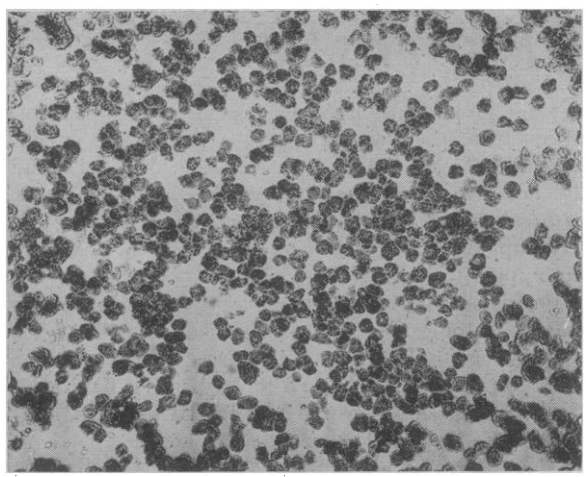

FIG. 1. Whole liver cells prepared from liver perfused with 0.027 *M* sodium citrate in calcium-free Locke's solution. Homogenization and purification carried out in calcium-free Locke's solution.

sum of the whole cells and the calculated broken cells.

The results obtained with preparations made from 25 rats are shown in Table 1. In the first two experiments shown (citrate), omission of back perfusion via the hepatic vein probably accounts for the low yield. Back perfusion with a pressure of 120 cm of water distends the liver considerably and appears to help separate the liver cells. However, similar back perfusion with complete Locke's solution did not increase the whole-cell yield. Although no extended experiments using rats of different ages have been made, it appears that older rats (350 g) give lower yields of whole cells.

Citrate, pyrophosphate, Versene, and ATP were all almost equally effective; glycerophosphate was less efficient in preventing cell breakage. Considerable variation in whole-cell yield in different preparations made with the same perfusion medium were noted.

Purified suspensions were prepared by sedimenting the whole cells by centrifuging 4 min at $110 \times g$ at 0° C, resuspending in calcium-free Locke's solution and resedimenting twice, using the same centrifugal field. A preparation prepared by perfusion with 0.027 *M* citrate is shown in Fig. 1.

The practical advantage of using suspensions of whole cells in place of slices are many and have been previously discussed by Elliott and Libet (*14*). It should be emphasized, however, that numerous alterations may well have occurred during the procedures described here, and the synthetic potentialities of such free cells may well differ considerably from those in the intact organ. It should be noted that Versene has recently been found to prevent the decline in oxidative phosphorylation otherwise seen in isolated heart mitochondria (*15*) and may have a similar effect here.

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Manuscript received October 17, 1952.

Purification and Crystallization of Hyperglycemic Glycogenolytic Factor (HGF)

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Murlin *et al.* in 1923 (*1*) first noted a hyperglycemic response obtainable with certain pancreatic extracts and suggested the name glucagon for the causative agent. Interest in material causing hyperglycemic action was increased by the finding that insulin preparations exhibited a hyperglycemic response which appeared as an initial and transient hyperglycemia following intravenous administration of the insulin preparation (*2, 3*).

Several workers (*2, 4*) have attempted unsuccessfully to isolate a highly purified material responsible for hyperglycemic action either from pancreas or from commercial amorphous insulin. In this communication we report the preparation of a novel crystalline compound (HGF) having a high degree of hyperglycemic activity.

Precautions were exercised in the isolation procedure to avoid steps involving denaturation or inactivation of contaminating proteins, principally insulin. In this way it was possible to use insulin assays to follow the removal of this particular contamination in various preparations made in the course of the work. The utilization of mild procedures also prevented decomposition of the hyperglycemic material.

The hyperglycemic activity was determined by noting the increase in blood sugar after intravenous injection of the test material into anesthetized cats. Blood sugar was determined on blood samples withdrawn at 5-min intervals during the first 25 min after the injection.

An amorphous fraction obtained during the com-

[1] We are pleased to acknowledge the assistance of H. J. Wick in providing the determinations of biological activities, and of H. A. Rose in photographing and characterizing the crystals.