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Mass Separation of Reticuloendothelial and Parenchymal Cells of Rat's Liver

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Recent studies (*1, 2*) have emphasized the importance of the hepatic reticuloendothelial (RE) system in the disposition of exogenously derived cholesterol. Consequently it appeared necessary to study these cells more closely than has been done.

A few years ago, Rous and Beard (*3*) were able to collect some RE cells by perfusing the liver of an animal injected with iron. The iron-containing RE cells present in the perfusate were isolated by means of an electromagnet. More recently, Anderson (*4*) described a method by which the total liver could be reduced to a suspension of its constituent cells. We believed these two different procedures, if combined, might offer a method for the separation and collection of relatively pure, viable hepatic parenchymal and RE cells in quantities sufficient so that the functions of each could be studied independently of the other. The method (*5*) described here achieved this separation.

The livers of rats that had been injected intravenously with 1 ml of a suspension of 15 percent pulverized carbonyl iron and 5 percent starch in saline on 3 successive days were perfused forward through the aorta and backward via the superior vena cava with isotonic calcium-sequestering fluid (1 part 0.11*M* versene, *p*H 7.4 to 9 parts Locke).

The entire liver was then removed and forced through a tissue press (garlic press) into a sufficient volume of fresh perfusion fluid to make a 3 to 4 percent suspension, stirred gently for about 10 min, and then poured through a 10 XX silk screen. Approximately 90 percent of the screen filtrate consists of intact liver cells.

The cell suspension was poured into conical siliconed centrifuge tubes and centrifuged at 45 *g* for 2 min. An upper, relatively clear supernatant layer (containing broken cells, vascular elements, and some of the non-iron-containing reticulocytes) was drawn off and discarded. The remaining two layers, the relatively cream-colored upper layer containing mostly hepatic parenchymal cells and the lower grey-black sediment containing RE cells preponderantly, were removed separately, resuspended in the perfusion fluid, centrifuged and separated as before, adding each layer to its respective counterpart obtained after the first centrifugation. This centrifuging washing procedure was repeated 3 times in all.

In order to obtain a pure collection of RE cells, the combined lower layers were suspended in 20 vol of a 4-percent solution of starch in isotonic saline containing 1 ml of 0.3 percent digitonin and 2 ml of Kreb's isotonic phosphate buffer per 100 ml. This suspension, although still containing some parenchymal cells, consists mainly of iron-containing RE cells.

The cell suspension was poured into a conical tube held upright in the field of a large eye magnet. The tube was rotated while being raised so that the magnetized cells described a helix as they were brought from the mouth to the apex of the tube. The fluid was then removed and resubmitted to the magnet as before. The procedure was repeated once more. All the sediments of iron-containing cells were combined, resuspended in the starch solution, and again brought to the bottom of the tube by the magnet. This resulted in a collection of RE cells of high purity (Fig. 1). In order to segregate hepatic parenchymal cells, the upper sedimentary layers obtained from the initial centrifugal process were suspended in perfusate and submitted to the magnet. The few iron-containing RE cells were quickly moved to the bottom of this nonviscous medium, and the upper layer on removal contained a morphologically homogeneous suspension of parenchymal cells (Fig. 2).

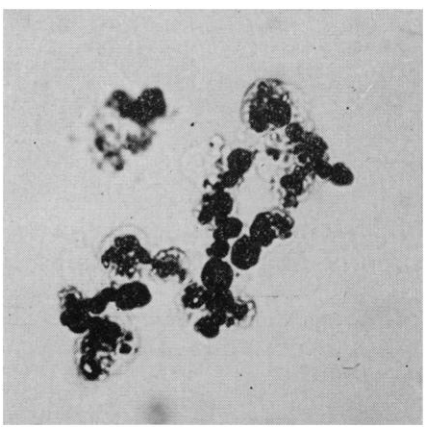

Fig. 1. Magnetized hepatic RE cells, unstained (× 800).

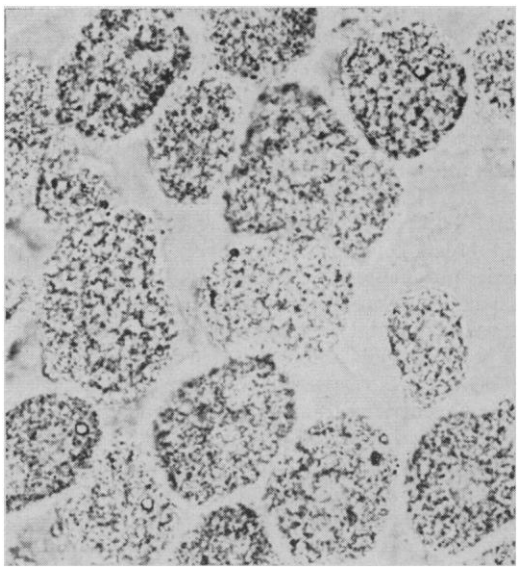

Fig. 2. Hepatic parenchymal cells from the same liver after separation from the RE cells, unstained (×800).

The afore-described procedure does not appear to destroy structural integrity of the cells, because neither cholesterol or biuret protein could be detected in the suspension fluid even after 4 hr of storage. Furthermore, the washed cells were observed, after the addition of ATP, to reduce triphenyl tetrazolium chloride at a normal rate.

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Effect of Maleic Hydrazide on the Respiration of Mature Onion Bulbs

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In a previous paper (*1*) the effects of aqueous sprays of maleic hydrazide on young, rapidly growing onion plants were presented. Results of enzyme assays indicated that maleic hydrazide reduced the dehydrogenase activity of the treated plants. The present paper (*2*) reports the respiratory effects of maleic hydrazide on stored mature onion bulbs harvested from plants previously treated with foliar applications of the hydrazide.

Sweet Spanish onions, Utah strain, were grown from seed and 14 wk after planting were treated with foliar sprays containing 500, 1000, 2000, 3000, and 4000 ppm of maleic hydrazide applied in the form of the diethanolamine salt at the rate of 0.16, 0.32, 0.64, 0.96, and 1.28 lb/acre of the free hydrazide. The mature bulbs were harvested 17 days after the hydrazide treatment, cured at room temperature for 2 wk, and placed in storage at 1°C for 23 wk.

After the storage period, it was observed that treatments of 3000 and 4000 ppm of maleic hydrazide greatly inhibited sprouting, whereas nontreated bulbs and bulbs treated with low concentrations of the hydrazide exhibited considerable sprouting.

Dehydrogenase activity and respiration as measured in a Warburg respirometer were determined on suspensions of onion bulbs minced in equal weights of phosphate buffer at *p*H 7.4. An anaerobic technique employing p,p′-diphenylenebis-2-(3,5-diphenyltetrazolium chloride), DBDTC, was used for the estimation of dehydrogenase activity (*3*). The colored reduction product was extracted with benzene and the optical density of the solution was read at 520 mμ. Oxygen absorption and carbon dioxide production were determined on the minced suspensions for 2 hr at 37.5°C.

At the end of the 23-wk storage period the effect of maleic hydrazide applications was evident, as indicated by the dehydrogenase activity and the respiration of the stored bulbs. The results of four determinations (Fig. 1) indicate that maleic hydrazide applications are stimulatory to respiration at low concentrations and inhibitory at high concentrations. An increase of the dehydrogenase activity and oxygen absorption could be obtained by the addition of succinate to those minces prepared from plants receiving low concentrations of the hydrazide. No stimulation of the enzyme system could be obtained by the

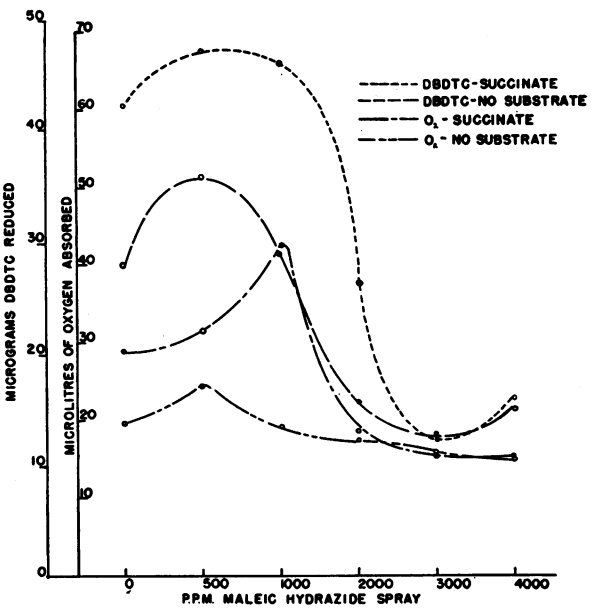

Fig. 1. Effect of maleic hydrazide on dehydrogenase activity and on oxygen absorption, as determined on 1 ml of minced onion tissue containing 58 mg of dry matter.