considerevolmente più elevati che nei controlli, mentre i livelli cerebrali di serotonina (5-HT) sono praticamente immodificati. La somministrazione di GH riduce i livelli di TP e 5-HIAA e non modifica i livelli di 5-HT sia nel topo nano che nel ratto ipofisectomizzato. Questi risultati dimostrano che la carenza di GH è un fattore importante nel determinare le alterazioni del metabolismo della 5-HT presenti in entrambi i modelli sperimentali.

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PRO LABORATORIO

An Instrument for Mechanical Dissociation of Tissues

Mechanical dissociation of tissues into single cells results to some extent in physical damage to cells. The aim of mechanical segregation is to obtain single cells without effects of chemical dissociating agents on cellular activity; in addition it serves to free certain tissue elements prior to further treatments. An appropriate dissociation technique is expected to yield quickly and conveniently large quantities of cells, most of which are undamaged and viable. Techniques commonly used for these purposes do not completely meet all of these requirements. Tissue mincing with scissors or razor blade, or squashing tissue fragments through a net, results only in a low rate of undamaged cells; dissociation of tissues with dissecting needles does not yield sufficient quantities of isolated cells within an adequately short period of time.

The difficulty is even more severe concerning separation of the covering layer of villi, as is the case with placental villous syncytiotrophoblast. There, the syncytial nature of the layer, its size and structure makes the task of obtaining considerable amount of whole undamaged cells very difficult.

In order to overcome these difficulties, special combs were devised for mechanical dissociation of tissues. A pair of these combs were used simultaneously in the process. The dissociation was performed by movement of one comb against the other with teeth interlocking, with the fragments of tissue lying between the two rows of teeth (Figure 1). The movement of the combs resulted in dissociation of the tissue pieces into individual cells or into very small tissue fragments, according to the type of tissue.

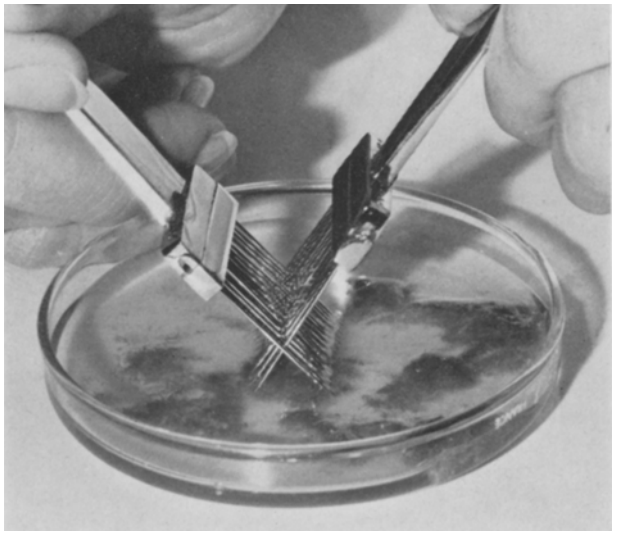

Fig. 1. The combs as used in the process of tissue dissociation.

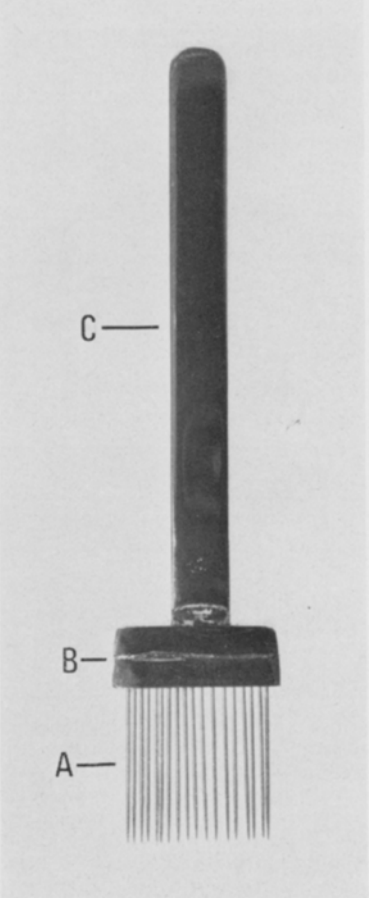

Fig. 2. A comb for tissue dissociation. A, needles; B, head; C, handle.

The comb is built of steel needles, set into a copper head on a long handle (Figure 2). Measurements of the needles are: length 25 mm, diameter 0.5 mm, tip diameter 50–150 μm (according to the nature of treated tissue). The head is 26 mm wide, 4 mm thick. The handle is about 120 mm long. A space of 0.5 mm was left between each two successive needles. The whole instrument is chrome-nickel plated and can be dry-air sterilized.

The combs were used successfully to free terminal villi and peeling syncytiotrophoblastic coverings from human term placentas, as the first step in a procedure developed in our laboratory for isolation of syncytiotrophoblast[1]. Recently the combs were used successfully to segregate lymphatic tissue from lymphatic organs in order to free individual lymphoid cells. Similarly conceived instrument was already used for teasing lymphocytes from lymph nodes[2]; however, this was built by 2 and 3 needles only and its efficiency was limited.

It seems that the described combs may be of help in dissociating procedures, where a quick separation of a larger amount of individual cells from flexible tissues is necessary.

Résumé. Des peignes spéciaux formés d'une rangée d'aiguilles insérées dans une poignée ont servi à dissocier des tissus. Ces peignes utilisés par paire on libéré rapidement de nombreuses cellules placentaires et lymphatiques intactes.

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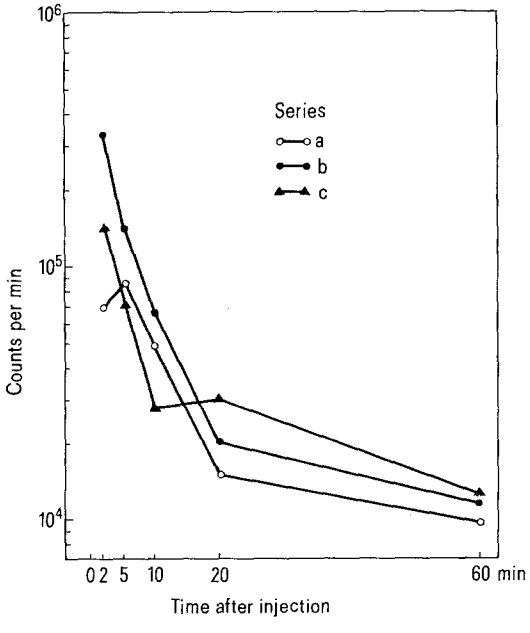
PRO EXPERIMENTIS

Factors Influencing the Serum Activity in Mice after Intravenous and Intraperitoneal Injection of ^{14}C Orotic Acid

Intravenous injection is easy to perform in the rat and gives reliable results. In smaller animals i.p. injection is often preferred, but a technique for i.v. injection into the tails of mice has been described[1]. During a study of orotic acid incorporation into mouse liver nucleotides and RNA, we found considerable amounts of the isotope left in the tails after injection into the tail vein[2]. We also found it difficult to judge the success of tail vein injections and wanted a more reliable criterium than visual examination. Furthermore, we found a 3–5-fold difference in serum activity between i.p. and i.v. injected mice. Due to the instability of the nucleotides during anoxia, blood had to be sampled in the peritoneal cavity after liver excision. Blood sampled in the peritoneal cavity seemed to be contaminated by the i.p. administered isotope up to 60 min after injection. In order further to evaluate the influence of injection technique and blood sampling technique on serum activity in mice, we have analyzed serum from blood sampled in the peritoneal cavity or from the brachial vessels 2 to 60 min after i.p. or i.v. administration of (6-^{14}C) orotic acid.



Cpm in total serum after injection of (6-^{14}C) orotic acid. Only the successfully injected animals (+ + +) from series c were considered. Semilogarithmic scale.

Materials and methods. Animals. Male NMRI mice (from Anticimex, Uppsala, Sweden) weighing 28–30 g were used. They were kept under constant conditions regarding light and temperature and were given standard food and water ad libitum.

Assay procedures. The isotope (6-^{14}C) orotic acid, spec. activity 61 mCi/m*M* (Amersham) was administered in 75 μl 0.9% NaCl (1.75 μCi) as a single 15 sec i.v. or i.p. injection. The injection of an exact volume of 75 μl was assured by use of a repeating dispenser (Hamilton Comp.). The animals were sacrificed at 2, 5, 10, 20 or 60 min after the injection. An oxygen-ether atmosphere was used to reduce tissue anoxia. Blood was collected either in the peritoneum after liver excision or from the brachial vessels before liver excision. The blood was coagulated and the radioactivity in the acid soluble fraction of the serum was determined in a Packard scintillator for 20 min. 3 alternative series were compared: a) i.p. injection combined with blood sampling from the brachial vessels; b) i.p. injection combined with blood sampling from the peritoneal cavity after liver excision; c) i.v. injection in the tail combined with blood sampling from the peritoneal cavity after liver excision.

The tails were hydrolyzed in 3 *M* KOH, and the radioactivity in each acidified hydrolysate was determined. Tails from tail vein injected animals were gently washed in water after blood coagulation at the site of puncture.

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