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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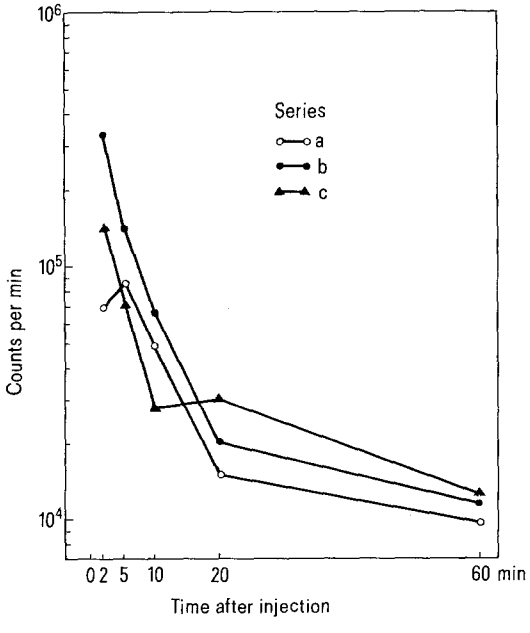
PRO EXPERIMENTIS

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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Cpm in serum and tails of tail vein injected animals (series [c]) and in tails of i.p. injected animals (series [a] and [b]).

Time after injection (min) [a]	Success rate [b]	cpm after i.v. injection ($\times 10^{-3}$)			cpm after i.p. injection ($\times 10^{-3}$)
		Serum [c]	Tail		Tail [d]
			Total	Washable	
2	+++	117	180	14	
2	+++	170	188	9	3–5
2	++	39	247	23	
5	+++	64	58	9	
5	+++	77	91	4	6–8
5	0	39	247	33	
10	+++	28	50	16	
10	++	51	242	12	7–9
10	0	32	247	22	
20	+++	32	30	4	
20	++	23	17	1	4–5
20	+	25	91	4	
20	0	42	203	56	
60	+++	14	41	13	
60	++	11	57	1	2–3
60	+	14	49	3	
60	0	12	78	29	

[a] The total dose injected was equivalent to 670×10^3 cpm. [b] +++, The isotope was clearly flushed along the vein and at withdrawal of the canula the vein was rapidly filled with blood. ++, After injection, vein filling was slower and a certain discolouration of the tail was noticed. +, Vein filling after injection quite slow and clear discolouration caused by a subcutaneous fluid buildup. 0, Very slow vein filling pronounced discolouration and outflow of isotope at the site of puncture. [c] 5.6 ml/100 g body weight[6]. [d] Highest and lowest values found at analysis of 6 animals.

The radioactivity of the water was measured to estimate the outflow of isotope from the vein to the outside of the tails at withdrawal of the canula.

Results and discussion. After tail vein injection large amounts of isotope remained in the tails and increased the radioactivity up to 50 times that found in tails from i.p. injected mice (Table). After i.p. administration, the isotope remained in the peritoneal cavity and increased the radioactivity of i.p. sampled blood serum as compared to serum collected fram the brachial vessels (Figure). When a similar amount of orotic acid was administered i.p. in 200 µl instead of in 75 µl, the contamination by unabsorbed isotope was still more pronounced[2]. Blood sampled from the brachial vessels of i.p. injected animals and blood sampled in the peritoneal cavity after tail vein injection should not be contaminated by local accumulation of isotope. The radioactivity found in these sera was in general quite similar. However, after 2 min the serum activity was higher after i.v. injection than after i.p. injection. This may indicate a more rapid isotope distribution after i.v. injection. After 20 and 60 min the serum activity was also higher after i.v. injection than after i.p. injection. At these stages the accumulation of isotope in the tails, still obvious at 60 min, may have contributed to an increased level of serum activity.

The attempt to judge the success of the tail vein injections by visual examination is shown in the Table. In general, injections which were considered successful resulted in high serum isotope levels and relatively low levels in the tails 2 and 5 min after administration. After 10 to 60 min, serum activities were also relatively high after unsuccessful injections. The high radioactivity in the tails of these unsuccessfully injected animals possibly functioned as a resevoir maintaining a proportionally high level of serum activity. It was possible to wash only a minor amount of the administered isotope from the tails of i.v. injected animals, proving that the isotope must have been trapped within the tail tissue.

Our results show that if the injection volumes are small, a substance may be administered either i.v. or i.p. in mice when a rapid distribution is desirable. Under circumstances where a slow uptake and distribution are favoured, the substance should be given i.p. in a relatively large volume. A slow uptake and distribution of the isotope can also be obtained after i.m. injection[3] or injection of isotope adsorbed onto activated charcoal[4]. Care must be taken not to have blood samples and peritoneal organs contaminated by local accumulations of the injected substance[2]. When anoxic effects are of no importance to the experimental results, blood sampling from the brachial vessels, from the retroorbital sinus, through cardiac puncture or by resection of the tail can be used. It is, however, important to have in mind that isotope activity might vary between sampling sites due to different tissue clearance capacities[5].

Zusammenfassung. ^{14}C-Orotsäure wurde mit zwei verschiedenen Injektionsmethoden in Mäuse injiziert und die Serumaktivität nach 2, 5, 10, 20 und 60 min gemessen. Nach i.p. Injektion wurde Blut, das aus der Bauchhöle nach Entfernung der Leber entnommen worden war, mit noch nicht absorbiertem Isotop gemischt. Bei i.v. Schwanzinjektion verblieb bei nicht perfekter Injektion eine erhebliche Menge der Orotsäure im Schwanzgewebe und ergab einen kontinuierlichen Isotopenzuschuss zum Serum.

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