Fixation for Electron Microscopy and the Retention of ^{3}H-Noradrenaline by Tissues

Localization of noradrenaline (NA) in tissue storage sites has been studied by radioautographic studies with ^{3}H-noradrenaline (^{3}H-NA)[1-3]. However, the preparation of tissues for electron microscopy could result in changes in the content or distribution of NA. In the present study, we have investigated the effect of fixation and dehydration procedures on tissue radioactivity (^{3}H) derived from ^{3}H-NA.

Methods. Four male albino rats (250–270 g) were anaesthetized with pentobarbitone and 250 μC (= 31 μg) of ^{3}H-NA was infused i.v. in each rat over a 15-min period causing a mean blood pressure rise of approximately 50 mm Hg. The animals were decapitated 5 min after the end of the infusion. Samples of heart, spleen, mesentery and vas deferens were taken for fixation. The remainder of each organ was frozen over solid carbon dioxide and analysed fluorimetrically for NA content[4]; total tissue radioactivity and ^{3}H-NA content were measured by liquid scintillation spectrometry. The tissue samples for fixation (cut into pieces of approximately 1 mm^3) were weighed and fixed in either (a) osmium tetroxide (1%) in 0.1 *M* cacodylate buffer (pH 7.4) (OsO_4), (b) glutaraldehyde (2%) -formaldehyde (2%) in 0.1 *M* cacodylate buffer (pH 7.4) (aldehyde) or (c) potassium permanganate[5] (3%) in phosphate buffer (pH 7.0) ($KMnO_4$). After (a) and (c), the tissue samples were dehydrated in alcohol; after (b) tissues were rinsed with buffer, post-fixed with OsO_4 and dehydrated in alcohol. The fixed tissues were hydrolysed with NaOH (2 *M*). All fixatives, washes and tissue hydrolysates were neutralized and decolorized with EDTA, H_2O_2 or oxalic acid, as required, and the radioactivity (^{3}H) of each was measured.

Reserpine (2 mg/kg s.c.) was given to 2 of the rats 18 h prior to the infusion of ^{3}H-NA.

Results and discussion. Tissues from control and reserpine-treated rats took up ^{3}H-NA to differing extents (Table I). The total ^{3}H content of the tissue samples taken for fixation (derived from the sum of the ^{3}H content of fixative solution, buffer washes, post-fixation and dehydration solutions and the hydrolysate of the fixed tissue) was comparable in most cases with the total ^{3}H content of the remaining unfixed tissue (Table I). This indicates that the tissue samples taken for fixation were representative of the tissue mass as a whole, and provides a reasonable basis for the comparison of the proportional distribution of ^{3}H between samples from fixed and unfixed tissues.

The percentage of ^{3}H retained in fixed tissue depended on the nature of the fixative and on the tissue used (Table II). With all fixation procedures, the radioactivity was found mainly in the fixation solutions and the fixed tissue and only a small proportion (less than 10%) was found in the buffer washes, post-fixation solutions and alcohols. After $KMnO_4$ treatment, less radioactivity was found in the fixed tissue than after OsO_4 or aldehyde fixation; this suggests that the ability of $KMnO_4$ to retain NA in tissues is much less than that of the other fixatives used.

The proportion of radioactivity retained by the tissue samples after fixation was much less in tissues from reserpine-treated rats than from control rats. In control and reserpine-treated tissues (Table II) there was an approximate relationship between the proportion of radioactivity present as ^{3}H-NA in the unfixed tissue and the proportion of radioactivity remaining in the fixed tissue. The relationship was closest after aldehyde fixation; after OsO_4 relatively more ^{3}H was retained in the fixed tissue, after $KMnO_4$ relatively less.

After reserpine, the percentage of ^{3}H present in unfixed tissues as ^{3}H-NA is reduced (Table II). This suggests that reserpine decreased the uptake of ^{3}H-NA and increased the metabolism of ^{3}H-NA. After OsO_4 fixation, some at least of these metabolites must have been retained in the

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Table I. Total noradrenaline (NA) and total radioactivity (^{3}H) content in unfixed tissue, and total ^{3}H content of samples of tissue taken for fixation (mean of experiments on 3 fixatives)

Tissue	Treatment	NA μg/g	Total ^{3}H in unfixed tissue cpm × 10^{-6}/g	Total ^{3}H in fixed tissue (mean for the 3 fixatives) cpm × 10^{-6}/g
Heart	C	1.1 1.1	2.3 2.0	1.9 1.8
	R	[a] [a]	0.8 0.8	0.7 0.6
Spleen	C	1.1 1.1	0.6 0.6	0.4 0.5
	R	[a] [a]	0.3 0.3	0.2 0.3
Mesentery	C	0.4 0.4	0.5 0.6	0.3 0.4
	R	[a] [a]	0.2 0.2	0.2 0.2
Vas deferens	C	13.0 9.0	0.5 0.6	0.2 0.5
	R	[a] [a]	0.5 0.5	0.4 0.4

Total ^{3}H content of fixed tissues was derived from the sum of the ^{3}H content of fixative solutions, buffer washes, post-fixation and dehydration solutions and the hydrolysate of the fixed tissue. Tissues from 2 control (C) and 2 reserpine-treated (R) rats were used.
[a] Not measurable.

Table II. The radioactivity (^{3}H) retained in the tissue after fixation given as a percentage of the total ^{3}H of the tissue sample

Tissue	Treatment	Percentage of ^{3}H remaining in tissues after fixation: OsO_4	Aldehyde	$KMnO_4$	Percentage of ^{3}H in unfixed tissues as ^{3}H-NA
Heart	C	72 86	66 61	63 40	63 65
	R	62 55	17 32	2 1	18 12
Spleen	C	66 58	53 50	35 30	57 49
	R	37 57	13 7	20 12	9 10
Mesentery	C	43 58	30 25	4 10	46 51
	R	17 29	5 8	9 10	5 8
Vas deferens	C	56 63	25 41	20 17	40 38
	R	13 31	17 17	11 12	10 14

The total was derived from the sum of the ^{3}H count in all fixatives, buffers, post-fixation solutions, alcohols and fixed tissue hydrolysate. The percentage of total radioactivity present as ^{3}H-NA in unfixed tissue is given for comparison. The rats used are the same as in Table I.

fixed tissue as the percentage retention of ^3H was greater than could be accounted for by ^3H-NA alone. After aldehyde fixation, the percentage retention of ^3H was similar to the percentage of ^3H present in unfixed tissues as ^3H-NA, which suggests that metabolites had been lost and only ^3H-NA retained by the fixation process. After $KMnO_4$ fixation, the percentage retention of ^3H was low so that in addition to the loss of metabolites, some at least of the ^3H-NA must have been lost. However, it is possible that with all the fixatives used, some of the ^3H in the fixed tissue is due to metabolites.

These experiments do not establish whether fixation results in the retention solely of ^3H-NA in tissues. The small size of the fixed tissue sample and the effects of the fixation process would make it almost impossible to determine chemically the form in which the ^3H is fixed. However, the good correlation observed between the percentage of ^3H present as ^3H-NA in unfixed tissues and the retention of ^3H by tissues after aldehyde fixation suggests that this fixation retains more NA and less metabolites than other fixatives; this is consistent with the specific histochemical affinity of glutaraldehyde[6] and formaldehyde[7] for catecholamines[8].

Résumé. Le dégagement de la radioactivité des tissus qui contiennent ^3H-NA et fixés pour la microscopie électronique est influencé par le genre des tissus (cœur, rate, mésentère, canal déférent) et par le fixatif utilisé (osmium tétroxyde, glutaraldéhyde-formaldéhyde, et permanganate de potassium). Les meilleurs résultats sont obtenus par la combinaison glutaraldéhyde-formaldéhyde, car les proportions de la radioactivité conservées dans ces tissus fixés sont similaires aux proportions de ^3H-NA dans les mêmes tissus non-fixés.

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Centrioles in Hepatocytes

Centrioles, which are small cytoplasmic bodies of about 150–200 nm in diameter and 300–350 nm in length concerned with organization of the spindle during cell division[1], have rarely been reported in adult mammalian liver cells. Bernhard and de Harven[1] observed a centriole in a parenchymal cell of a mouse in which the liver was infiltrated with leukemic cells but noted that the hepatocytes were showing regenerative activity. They pointed out that 'dans la cellule hépatique et dans les cellules tubulaires rénales de Mammifères, le centriole a été décrit en microscopique optique. Il est donc surprenant de constater que les spécialistes electroniciens de ces deux tissus ne semblent pas y avoir observé de centriole'. David[2] mentioned that he found centrioles in liver cells of guinea-pigs recovering from prolonged fasting as well as in embryonic liver cells[3] but states that 'this structure has never been described from normal liver cells'[2]. More recently Afzelius and Schoental[4] reported the occurrence of 3 centrioles within a liver cell of a weanling rat in which the liver had been damaged by retrorsine treatment. They considered this finding of significance because of the lack of documentation of centrioles in *normal* liver cells.

In our laboratory, liver was included as one of a number of different tissues from young adult rats in an electron microscopic survey for 9 + 0 cilia[5–7]. In tissues in which cilia were not readily apparent it was necessary to establish their absence by examining closely the centrioles of many cells since it is from these organelles that cilia take their origin. This communication, which is a retrospective study of findings in the rat liver, has 2 purposes, (1) to show that centrioles are commonly found in normal as well as regenerating hepatocytes of the rat, and (2) to demonstrate that more than 2 centrioles can occur in a single hepatocyte in normal liver. The results also indicate that cilia are not associated with centrioles in hepatocytes of normal and regenerating liver.

Small pieces of liver were taken from 3 normal 60-day virgin female Sprague-Dawley rats which had received no treatment of any kind, and from groups of 3 rats killed 22, 48 and 72 h after partial ($^2/_3$) hepatectomy. They were fixed in veronal-buffered osmium tetroxide[8] for 2 h at 4 °C, dehydrated in absolute ethanol and embedded in Epon 812[9]. Sections were cut with glass knives on a Cambridge Huxley or LKB ultramicrotome usually at 600–900 Å. They were stained with lead[10] before being mounted on uncoated copper grids and being examined in a Siemens Elmiskop 1 electron microscope at an accelerating voltage of 80 kV.

Normal liver. Centrioles are present in normal rat hepatocytes. Electron micrographs of 23 centrioles were taken during the survey, this representing not more than a third of the total number of centrioles found. Of the 23 examples analysed in retrospect, 17 showed 1 centriole in the plane of section, 6 in T.S. (Figure 1), 4 in L.S. (Figure 2) and 7 in oblique section. Five of the remaining electron micrographs showed 2 centrioles (diplosomes) in

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