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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the sections and the last electron micrograph showed 3 closely associated centrioles in a hepatocyte (Figure 3). The centrioles, measuring 150–200 nm in diameter and 300–400 nm in length, were typical of mammalian centrioles in general, being comprised on 9 sets of triplet fibres forming the walls of a hollow tube. No evidence of centriolar replication was found in normal hepatocytes.

In 1 hepatocyte in the above series, the centriole showed radiating processes from the triplet fibres, giving the appearance of the attachments a centriole makes with a membrane in the formation of a basal body. However, no more definite evidence of basal body formation was observed and ciliary development was not seen.

Regenerating liver. Centrioles were found in hepatocytes during the intense regenerative activity which follows partial hepatectomy at each of the intervals examined. The increased size of the regenerating hepatocyte and its tendency to accumulate large amounts of lipid made it more difficult to detect centrioles than in the normal liver cell. In the hepatocytes examined 22 h after partial hepatectomy, i.e. before mitotic activity became readily apparent, centriolar replication was found (Figure 4) as in hepatocytes at 48 and 72 h after operation.

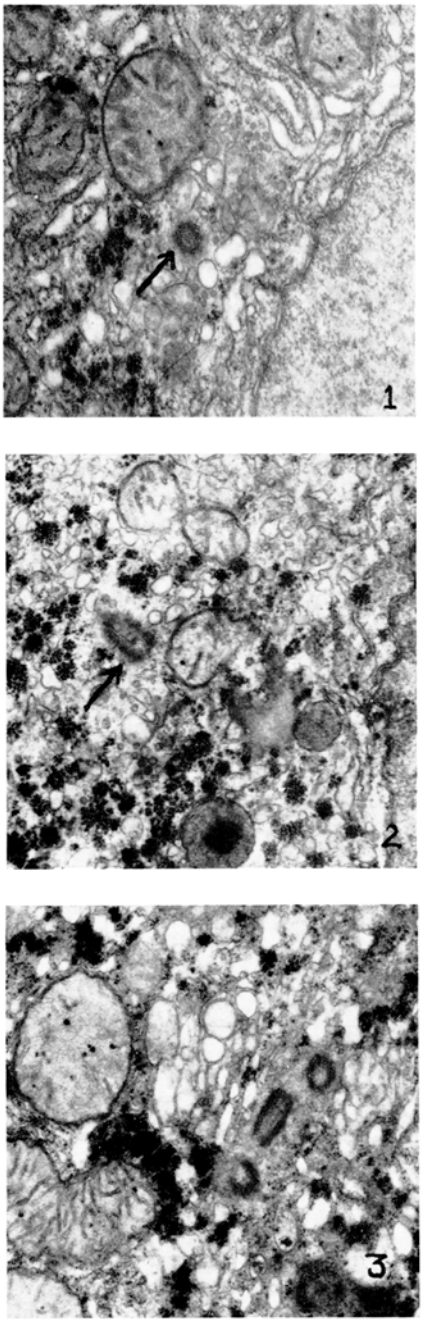
No evidence of cilium formation from the centrioles of regenerating liver cells was found.

The hepatocytes of both the normal and regenerating liver of young adult rats possess centrioles. In size and structure they are identical with centrioles in other mammalian cells. Since 6 of the 23 electron micrographs analysed from the normal liver included more than 1 centriole per hepatocyte, a ratio similar to that found in other tissues, it seems probable that hepatocytes usually possess diplosomes (paired centrioles). Evidence of more than 2 centrioles in hepatocytes has been found; in view of the high incidence of binucleate cells (25–35% of the hepatocytes) and polyploidy in the liver of the 60-day rats that have been studied, the presence of more than 2 centrioles is not entirely surprising.

The apparent absence of daughter centrioles suggests that replication rarely occurs in the normal hepatocyte. This would agree with the very low mitotic activity (considerably less than 1 cell $^0/_{00}$) found in the hepatocytes of the normal 60-day rats used in this study. In contrast, after the stimulus of partial hepatectomy, centriolar replication can frequently be observed (Figure 4).

Although mitotic activity of cells in the normal adult rat liver is relatively infrequent, there is a very slow turnover of cells. The significance of the present observations is to establish beyond doubt that normal hepatocytes possess fully differentiated centrioles and therefore have the necessary basic apparatus for organizing the mitotic spindle. A potent mitogenic stimulus such as partial hepatectomy induces centriolar replication in preparation for further cell divisions.

In view of the large number of ultrastructural studies on normal rat liver, it is surprising that centrioles have not been reported. There are a number of possible ex-



Figs. 1–3. Young adult rat liver, fixed in Palade's veronal buffered osmium tetroxide, contrasted with lead stain. Fig. 1. T.S. centriole. × 18,500. Fig. 2. L.S. centriole. × 26,000. Fig. 3. Section containing 3 centrioles. × 18,500.

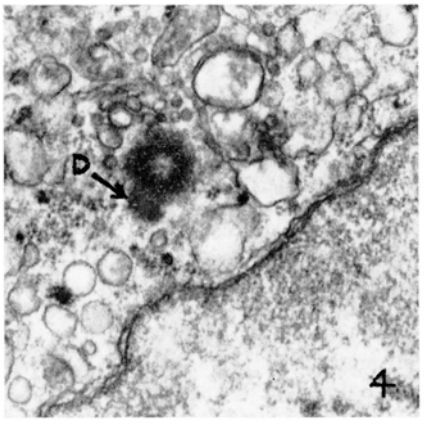


Fig. 4. Young adult rat liver 22 h after partial ($^2/_3$) hepatectomy, fixed and stained as for Figures 1–3. A centriole in T.S. is seen budding a daughter centriole. × 37,000.

planations. Whereas many other workers have had their attention drawn to the multiplicity of ultrastructural features of hepatocytes, in the present study attention was only paid to the detection of centrioles. Although this may in part account for the disparity, other factors are probably involved. Firstly, because of the very large size of the mature hepatocyte and its small nucleo-cytoplasmic ratio, the chances of cutting the centrioles is far less than in most other cell types. Secondly, the liver cell nucleus does not have a discernible hilar region where centrioles are usually found as, for example, in lymphocytes; in the hepatocyte, therefore, attention is not drawn to the area most likely to contain the centrioles. Finally, hepatocytes contain many small vesicular bodies, densely staining glycogen granules, lipid droplets, and lysosomes which tend to obscure the less densely staining centriole (as in Figure 2).

Sufficiently large samples of centrioles in both normal and regenerating liver have been examined to establish the improbability of hepatocytes possessing cilia. Cilia have been detected, however, in capillary endothelial cells (unpublished observations) and in bile duct epithelial cells[11,12] in the rat liver[13].

Zusammenfassung. In der normalen und regenerierenden Rattenleber wurden Zentriolen gefunden. In der normalen Leberparenchymzelle erscheinen sie wahrscheinlich als Diplosomen. Häufig werden mehr als 2 Zentriolen in der einzelnen Leberparenchymzelle beobachtet, jedoch gibt es keine Anhaltspunkte für eine zentrioläre Replikation in den normalen Leberparenchymzellen. Teilhepatektomie tritt kurz nach Bildung von Tochterzentriolen auf. Das Vorkommen von Zilien scheint mit den Zentriolen der Leberparenchymzellen nicht verbunden zu sein.

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Catecholamine-Containing Nerve Fibres in the Hind-Gut of the Crayfish *Astacus astacus* L. (Crustacea, Decapoda)

A number of investigations have been devoted to the intestinal nervous system of *Astacus astacus*[1-3]. By means of the specific fluorescence method of FALCK and HILLARP[4] for the cellular localization of certain monoamines, we were able to demonstrate the presence of monoaminergic fibres in this part of the nervous system. In a previous investigation, the central nervous system of *A. astacus* was described according to this method[5].

There is full agreement in the previous investigations that the innervation of the crayfish intestine derives from 2 main sources; one, the ganglia of the circumesophageal connectives that innervate the anterior part of the intestine; the other, the last abdominal ganglion that innervates the hind-gut. From the last abdominal ganglion in the crayfish, 1 nerve turns dorso-caudal to the hind-gut where it divides into 1 posterior and 2 anterior branches. These give rise to a dense network of fibres, the 'Grundplexus' of ALEXANDROWICZ[1] (p. 403), surrounding the whole hind-gut. Without sharp limits, it continues as a second order plexus, called by ALEXANDROWICZ 'Endplexus', which innervates the muscles. The fibres of this latter plexus are parallel to the muscles in contrast to the 'Grundplexus' where the fibres are randomly distributed. The circular muscle layer is rather thick. The longitudinal muscles, confined to the 6 ridges of the intestine, are concentrated in a few bundles. Their special mode of insertion is described, among others, by ALEXANDROWICZ[1].

There are some statements in the literature with special regard to catecholamines in crustaceans. ÖSTLUND[6] testing *Daphnia pulex* and *Crangon crangon* and v. EULER[7] working on *Carcinus maenas* could not demonstrate any appreciable amounts of adrenaline and noradrenaline in extracts of whole animals. Recently KERKUT, SEDDEN and WALKER[8] reported the presence of dopamine in the thoracic nerve mass of *C. maenas* in a concentration of 7.3 µg/g. They found no adrenaline or noradrenaline. FLOREY[9] found that adrenaline and noradrenaline were active on the hind-gut of the crayfish *Cambarus clarkii* increasing contraction height. However, he concluded that this effect was not specific and suggested that the compound active on the gut was of a structure similar to adrenaline or noradrenaline.

In the present experiment fibres emitting a green fluorescence, characteristic for catecholamines, were observed in both plexa mentioned above, whereas no fibres emitting a yellow fluorescence, which would indicate the presence of 5-hydroxytryptamine, were found. The green fluorescent fibres seem to constitute only a minor part of the plexa, which according to ALEXANDROWICZ contain a large amount of fibres. The fluorescent fibres are found among both the circular and the longitudinal muscle fibres. In the circular muscle layer, they are more abundant in the outer part, the 'Grundplexus', where they are thicker and of a less typical beaded appearance than those of the 'Endplexus'. In the latter, they appear as thin threads with numerous varicosities (Figure). Among the muscle fibres, sparsely distributed bulb-shaped structures are also found. They contain several green fluorescent granules. It is not known whether these represent terminations of the varicose fibres. Besides the green fluorescent

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