

# Effects of hyperthermia therapy on the liver

## II Morphological observations

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**SYNOPSIS** Liver biopsy specimens obtained from three patients during treatment of advanced malignant disease by exogenous hyperthermia were studied by light and electron microscopy. In two patients the parenchymal cells showed either slight swelling or nuclear alterations by light microscopy and, at the fine structural level, large numbers of autophagic vacuoles, dilatation of Golgi elements and endoplasmic reticulum, and large cytoplasmic vacuoles. Similar changes, but in much more severe form together with parenchymal cell necrosis and cholestasis, were seen in the liver of case 3, who developed jaundice 24 hours after hyperthermia. The findings are discussed in relation to earlier accounts of liver changes following hyperthermia, and the functional implications are considered.

In the course of evaluating liver function during treatment of advanced malignant disease by exogenous hyperthermia (Findlay *et al*, 1975) liver biopsy specimens were obtained from three patients. We report here the structural changes observed by light and electron microscopy.

management is presented elsewhere (Findlay *et al*, 1975), but a brief clinicopathological summary, together with details of the hyperthermia technique and times at which Gillman needle biopsy specimens of liver were obtained, is given in the table. Case 3 was not included in the BSP studies, but cases 1 and 2 were numbers 7 and 2 respectively in the other series (Findlay *et al*, 1975).

### Material and Methods

#### PATIENTS STUDIED

A more complete account of the patients and their <sup>1</sup>Correspondence and address for reprints: Dr E. J. Wills, Clinical Research Centre, Watford Road, Harrow HA1 3UJ. Received for publication 15 July 1975.

#### SPECIMEN PREPARATION

The major part of each specimen was fixed in 10% formol saline and prepared for light microscopy by conventional techniques. Paraffin sections were stained with haematoxylin and eosin (H and E),

Case No.	1 Osteogenic sarcoma			2 Carcinoma stomach			3 Malignant melanoma		
Diagnosis	None			Skin, mediastinum			Skin		
Metastasis									
Serum bilirubin (0.2-0.8 mg/100 ml) <sup>1</sup>									
Pre, at, and post hyperthermia	0.3	0.2	0.3	0.3	0.1	0.2	0.6	—	8.5 <sup>2</sup>
Alkaline phosphatase (20-85 IU) <sup>1</sup>									
Pre, at, and post hyperthermia	58	49	52	112	85	83	34	—	63
SGOT (4-20 IU) <sup>1</sup>									
Pre, at, and post hyperthermia	13	15	13	24	14	24	—	—	—
Duration at temperature (min)									
> 40°C	125			65			495		
> 41°C	—			—			430		
> 41.5°C	—			—			410		
> 41.8°C	—			—			390		
> 42°C	—			—			190		
Time of biopsy	Before, immediately following and 24 hours after hyperthermia			Before and 24 hours after hyperthermia			2 days after hyperthermia		

Table Summary of cases studied

<sup>1</sup>Normal range.

<sup>2</sup>Serum bilirubin rose to 18.5 mg/100 ml on the 5th day after hyperthermia, and had returned to normal by 4 weeks.

periodic acid-Schiff (PAS) with and without diastase digestion, Gordon and Sweet's method for reticulin, and the haematoxylin-van Gieson and Perl techniques.

The portion of each sample for electron microscopy was cut into small blocks which were fixed in chilled cacodylate-buffered 3% glutaraldehyde solution (Sabatini *et al.*, 1963) for 1 to 4 hours then washed in 10% buffered sucrose solution for 1 hour. After post-fixation with 1% osmium tetroxide in phosphate buffer (Millonig, 1961) for 1 hour the specimens were dehydrated in a graded series of alcohol solutions followed by propylene oxide and embedded in epoxy resin (Araldite, CIBA). One-micron sections were stained with 1% toluidine blue in 1% aqueous borax solution for light microscopy. Ultrathin sections for electron microscopy were contrast stained with alcoholic uranyl acetate (Watson, 1958) followed by lead citrate (Reynolds, 1963) and examined in an AEI EM6B electron microscope.

## Results

### LIGHT MICROSCOPY

#### Case 1

Before hyperthermia there were a few foci of non-specific reactive cells. Parenchymal cell nuclei were pleomorphic and one mitosis was seen. No change was apparent at the end of the hyperthermia period, but after 24 hours the parenchymal cells showed slight, but generalized, swelling (fig 1).

#### Case 2

An occasional mitotic liver cell was noted before hyperthermia, together with an excess of polymorphs within the sinusoids. Twenty-four hours after treatment the liver cell nuclei varied in size and contained enlarged nucleoli, lipid vacuoles, and PAS-diastase positive eosinophilic inclusions which were sometimes limited by a membrane identical with the nuclear envelope. A few acidophilic bodies were also observed.

#### Case 3

In the single specimen obtained two days after hyperthermia there were large numbers of pale swollen parenchymal cells which showed clear areas in the cytoplasm (fig 2) and were depleted of glycogen. Many contained lipid vacuoles, together with round or ovoid inclusions which were stained pale pink by H and E (fig 2), PAS and PAS-diastase, and sometimes contained tiny dense PAS-diastase positive peripheral granules. In epoxy sections after toluidine blue (fig 3) these inclusions were a faint blue which was readily distinguishable from the slightly greenish tinge of lipid droplets. In addition, irregular dense purplish cytoplasmic patches were frequently apparent (fig 3). Many nuclei had undergone the

same changes as in case 2 after hyperthermia (figs 2 and 3). An occasional acidophilic body (fig 3) and focal reticulin collapse with attendant accumulations of round cells provided evidence of liver cell necrosis. There was mild, predominantly centrilobular, cholestasis (fig 3), and increased numbers of polymorphs and round cells were found in portal tracts and sinusoids (fig 2).

### ELECTRON MICROSCOPY

#### Case 1

Parenchymal cells were normal before hyperthermia but many Kupffer cells were enlarged and showed increased phagocytic activity. The focal aggregations of lymphocytes, macrophages, and occasional plasma cells observed by light microscopy lay within the sinusoidal lumina.

Immediately following hyperthermia the parenchymal cells contained large numbers of early autophagic vacuoles within which the enveloped cytoplasmic organelles were still easily recognizable (fig 4). Golgi vesicles and cisternae were frequently dilated (fig 4).

Twenty-four hours after hyperthermia the autophagic vacuoles were still apparent but mostly now in residual body form (fig 5). Golgi swelling was more marked (fig 5), and in scattered cells the endoplasmic reticulum was moderately dilated and filled with flocculent material of low electron opacity. A few large vacuoles of the type described in case 3 were also seen, and isolated mitochondria were pale and swollen.

#### Case 2

In the pre-hyperthermia specimen occasional hepatocytes showed dilatation of their endoplasmic reticulum, or the nuclear and cytoplasmic condensation of early acidophilic necrosis. Autophagic vacuoles and residual bodies were numerous and Kupffer cells were actively phagocytic.

Considerably more autophagic vacuoles, most of them large and in an early developmental stage, were noted in parenchymal cells after hyperthermia. Golgi elements were occasionally dilated, and in a few cells the endoplasmic reticulum was greatly distended.

#### Case 3

Parenchymal cell nuclei contained lipid droplets, cytoplasmic pseudoinclusions or flocculent material which was enclosed usually by a single membrane but sometimes also in part by paired membranes similar to the nuclear envelope (fig 6). Many nucleoli were enlarged with expansion of the skein-like nucleolonema (fig 6).

In addition to the changes in Golgi elements and

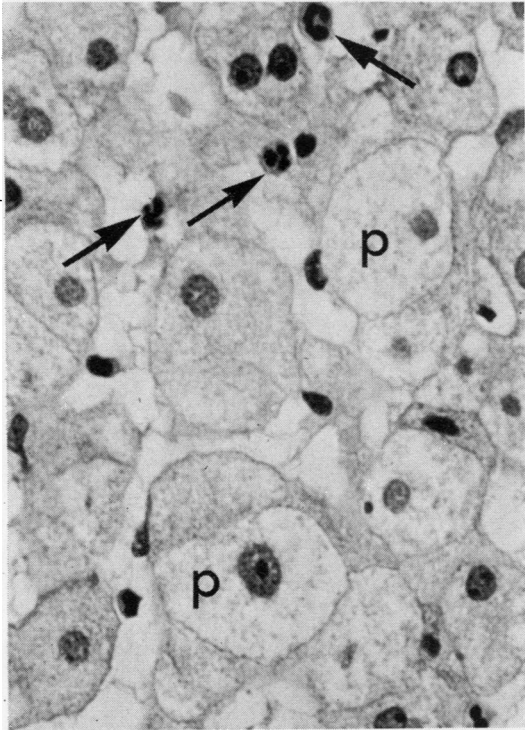


Fig 1

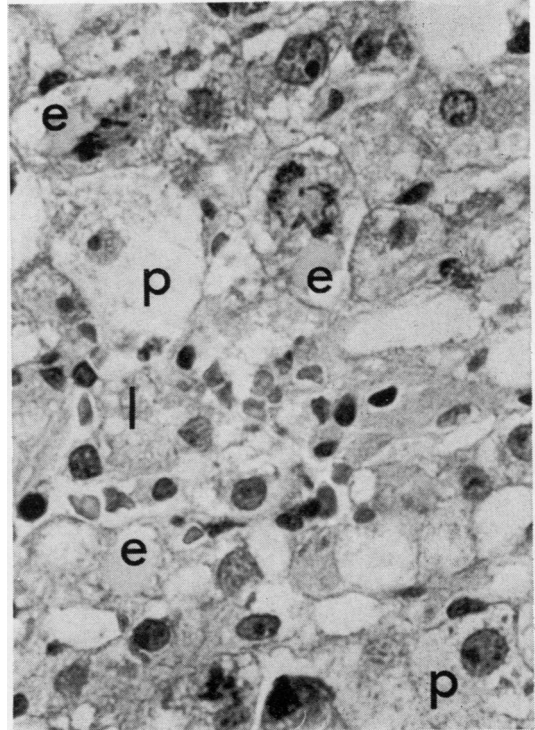


Fig 2

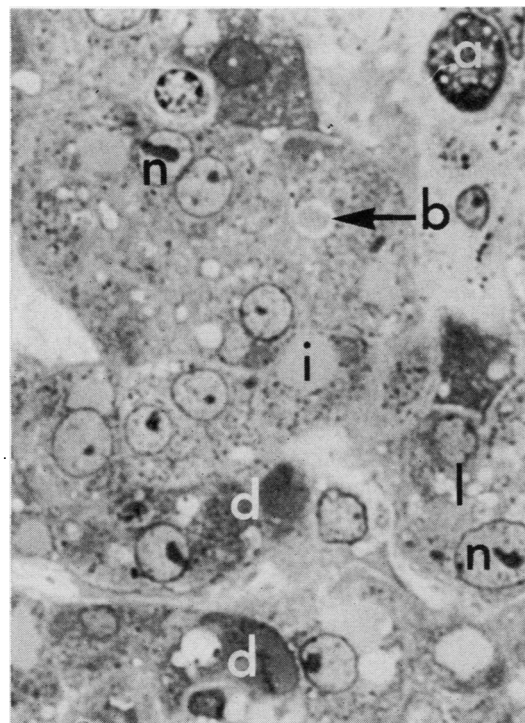


Fig 3

Fig 1 Case 1, 24 hours after hyperthermia. There is swelling and pallor of parenchymal cells (p). Sinusoids are narrow and contain excess numbers of polymorphs (arrows). Haematoxylin and eosin  $\times 720$ .

Fig 2 Case 3, 2 days after hyperthermia. Some of the parenchymal cells are swollen and pale (p), others contain lipid vacuoles (l) or pale eosinophilic inclusions (e) in their cytoplasm; the nuclei of two of these cells are in mitosis. Increased round cells are present at the centre of the field. Haematoxylin and eosin  $\times 720$ .

Fig 3 Case 3, 2 days after hyperthermia. There are large, dense, irregular areas (d), lipid vacuoles (l), and pale rounded inclusions (i) within the cytoplasm of parenchymal cells, and enlargement of their nucleoli (n). A pigment plug within a bile canaliculus is seen (b) and an acidophilic-type body (a). Araldite section, toluidine blue  $\times 960$ .

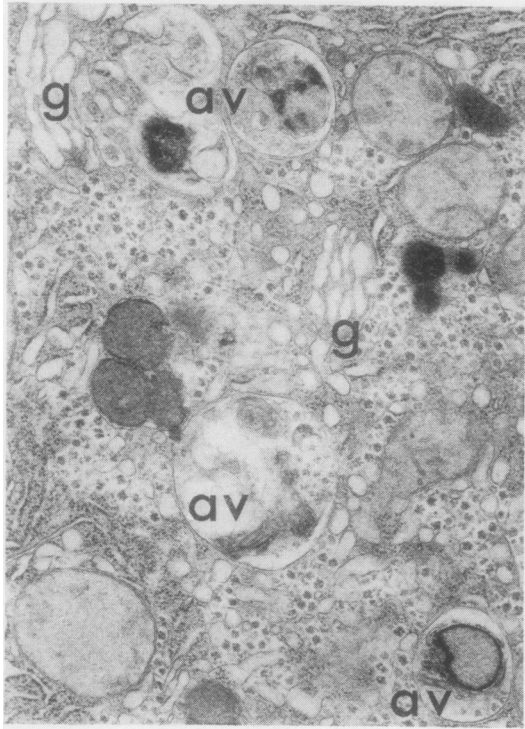


Fig 4

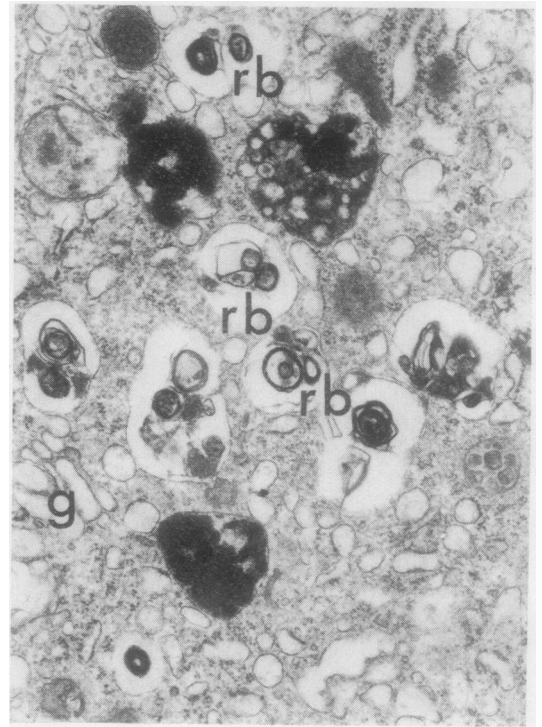


Fig 5

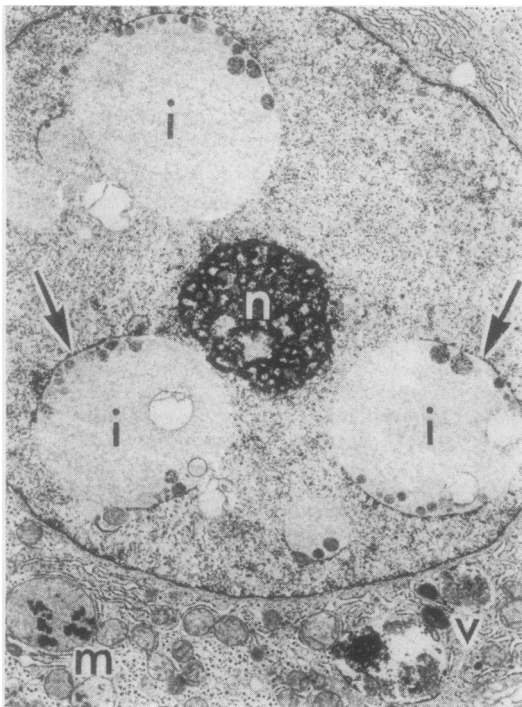
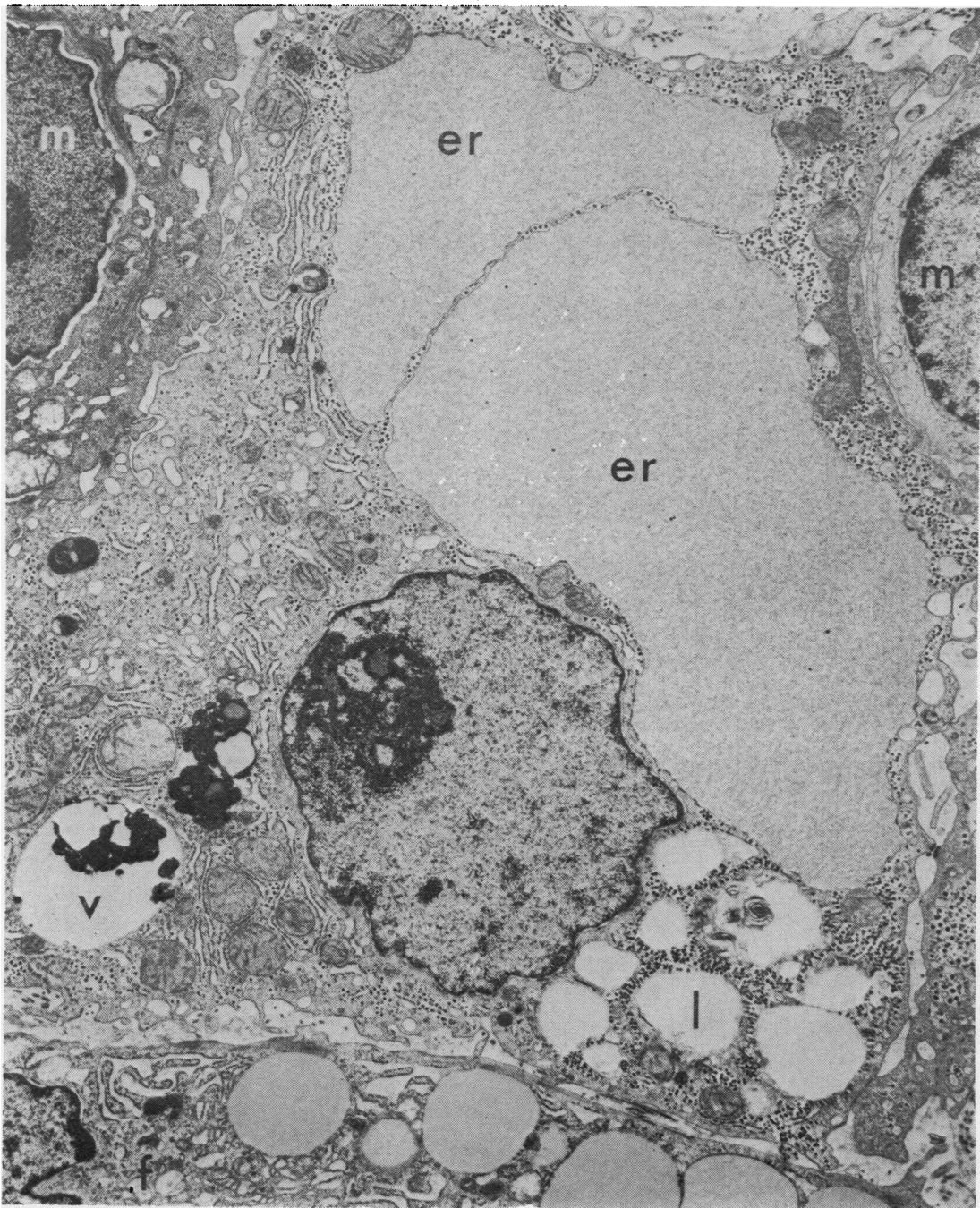


Fig 6

**Fig 4** Case 1, immediately following hyperthermia. Portion of a parenchymal cell showing autophagic vacuoles (av) in which enveloped organelles are recognizable. There is dilatation of Golgi elements (g).  $\times 20\ 000$ .

**Fig 5** Case 1, 24 hours after hyperthermia. Autophagic vacuoles in the parenchymal cell cytoplasm are mostly at the residual body stage (rb). Golgi components (g) are dilated.  $\times 20\ 000$ .

**Fig 6** Case 3, 2 days after hyperthermia. A parenchymal cell nucleus contains an enlarged nucleolus (n) and multiple inclusions (i) bounded in part by nuclear membrane (at arrows). In the adjacent cytoplasm dense material is present within two mitochondria (m), and two vacuoles (v) show greater contents.  $\times 6000$ .



**Fig 7** *Case 3, 2 days after hyperthermia. Survey of a parenchymal cell with massive dilatation of two rough-surfaced cisternae of endoplasmic reticulum (er), a large cytoplasmic vacuole (v), and lipid droplets (l). At the top two macrophages (m) have infiltrated along sinusoidal recesses; a fat-storing cell (f) lies at the bottom of the field.  $\times 10\ 000$ .*

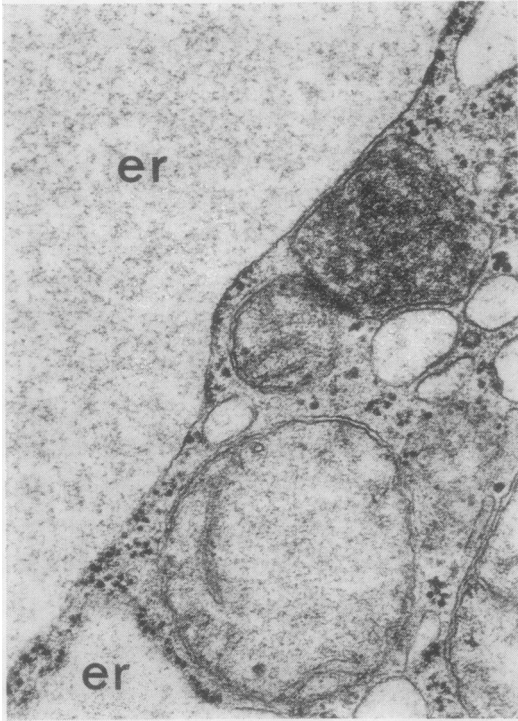


Fig 8

Figs 8-10 Case 3, 2 days after hyperthermia.

Fig 8 Detail of a parenchymal cell showing finely granular material within dilated cisternae of endoplasmic reticulum (er) which have lost many of their attached ribosomes.  $\times 46\ 000$ .

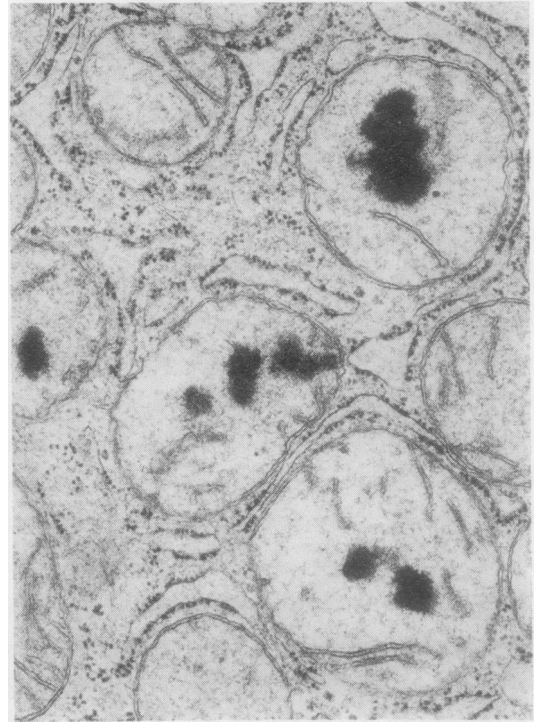


Fig 9

Fig 9 Parenchymal cell mitochondria containing irregular electron opaque deposits.  $\times 30\ 000$ .

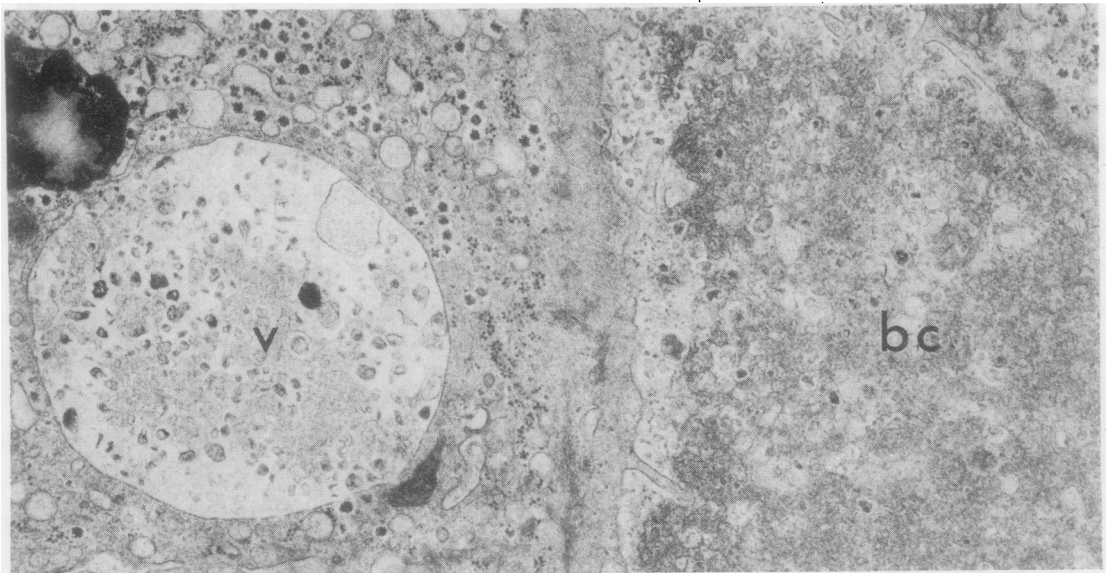
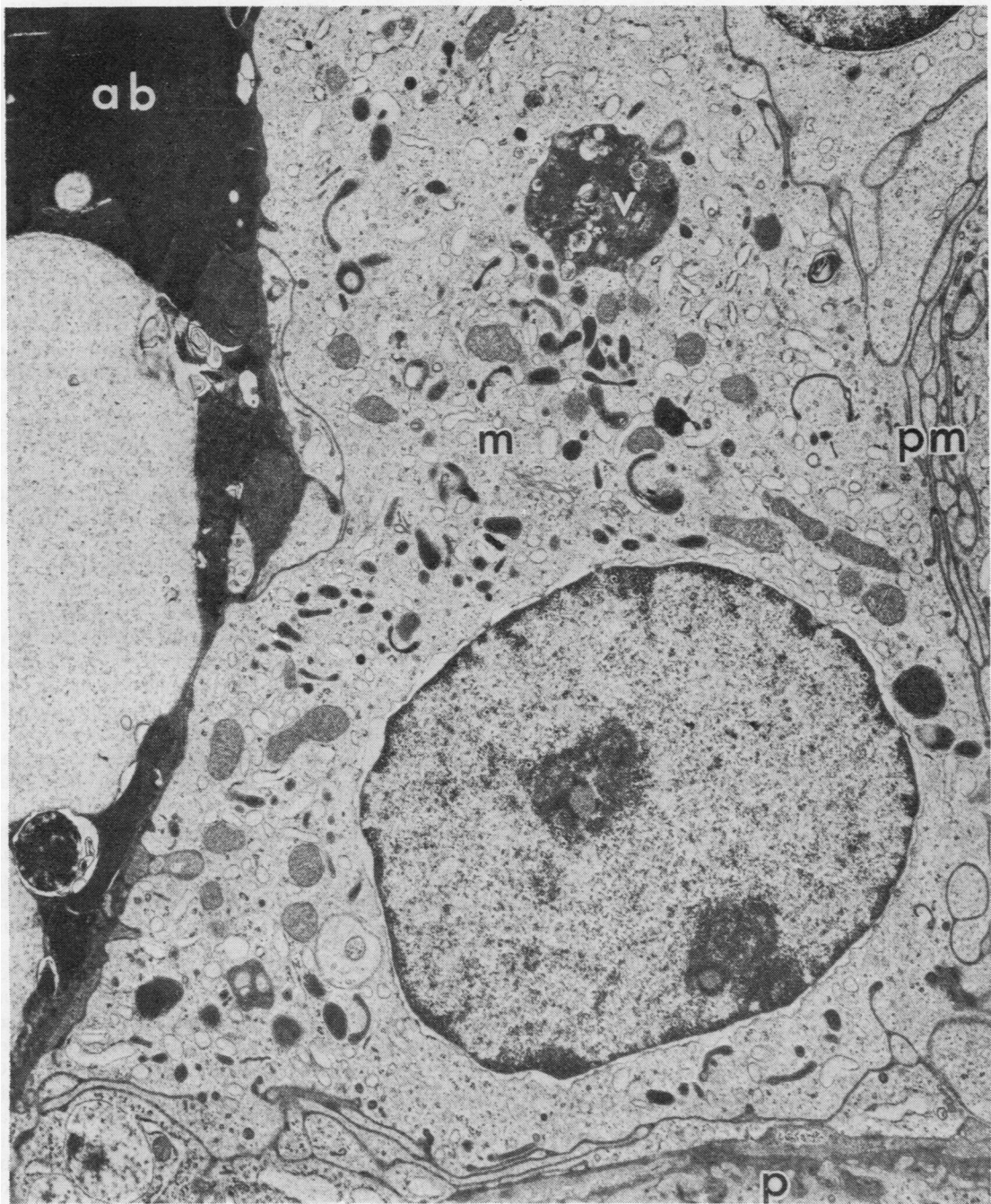


Fig 10 A large cytoplasmic vacuole (v) is present in a parenchymal cell. To the right is part of a grossly dilated bile canaliculus (bc); the microvilli have disappeared and the lumen is filled with a plug of bile pigment.  $\times 24\ 000$ .



**Fig 11** *Case 3, 2 days after hyperthermia. Part of a sinusoid filled with inflammatory cells. Most of the field is occupied by a large macrophage (m), which shows complex foldings of the plasma membrane (pm) and a dense cell remnant in a phagocytic vacuole (v). An acidophilic body (ab) is also present. Part of a parenchymal cell (p) is seen at the bottom of the field.  $\times 10\ 000$ .*

rough-surfaced endoplasmic reticulum seen in cases 1 and 2, a number of cells contained a few massively distended cisternae (figs 7 and 8). In such cells the remaining rough cisternae, although normal in profile, displayed focal loss of ribosomes. Smooth-surfaced endoplasmic reticulum was little altered. As well as the mitochondrial swelling and pallor described in the other subjects there were occasional irregular, intensely electron opaque deposits which replaced the normal mitochondrial dense granules (figs 6 and 9).

Cytoplasmic lipid droplets were numerous (fig 7), as were autophagic vacuoles. There were also many large vacuoles up to  $12\ \mu$  in diameter (figs 6, 7, and 10) bounded by a smooth, sometimes discontinuous, membrane, which either appeared empty or contained wispy material mixed with a few small non-descript granules or myelin figures (fig 10), glycogen particles, and lipofuscin granules (fig 7). Bile pigment was frequently deposited within the parenchymal cell cytoplasm and plugged the lumen of bile canaliculi which were dilated and had lost their microvilli (fig 10). Condensation of hepatocytes, progressing to the formation of free acidophilic type bodies within sinusoids (fig 11), was seen; this type of change was sometimes combined with autophagocytosis and massive dilatation of the endoplasmic reticulum.

Many sinusoids were obstructed by enlarged Kupffer cells and macrophages with complex foldings of their plasma membranes, numerous dense body lysosomes, and phagocytosed cell remnants in their bulky cytoplasm (fig 11). Aggregations of neutrophils, lymphocytes and an occasional plasma cell in such areas were in close apposition to hepatocytes.

Occasional intrahepatic bile ducts showed a mixture of 'dark' and 'light' cell changes often accompanied by large autophagic vacuoles. Focal loss of microvilli and the formation of large blebs at the apical surface of biliary epithelial cells were also observed.

## Discussion

In the pre-penicillin era fever therapy was an established mode of treatment for neurosyphilis, sulphonamide-resistant gonorrhoea, and a number of other diseases. Jaundice, appearing on the second or third day after hyperthermia and usually of brief duration, was considered an infrequent side effect, although in MacDonald's (1944) series the incidence was 19%. Furthermore, transient hyperbilirubinaemia was detected in three-quarters of the patients studied by King *et al* (1943) and in all those investigated by Wallace and Bushby (1943). Descriptions

of the liver lesions were of necessity restricted to necropsy material from the occasional fatalities which occurred, and initial reports simply noted liver necrosis in association with generalized tissue congestion and haemorrhages (Hartman and Major, 1935; Chunn and Kirkpatrick, 1937; Wilbur and Stevens, 1937).

A more detailed account of 17 fatal cases from the Army Institute of Pathology, Washington was published by Gore and Isaacson (1949). In the early post-hyperthermia period, there was liver congestion with cloudy swelling and glycogen depletion of parenchymal cells, followed by nuclear and cytoplasmic vacuolation and the accumulation of fat droplets. Those patients who died more than 48 hours after hyperthermia had all developed clinical jaundice and their livers showed centrilobular necrosis which became increasingly severe as the survival period lengthened. By seven days actively phagocytic macrophages, together with regenerating hepatocytes and bile duct proliferation at the lobular periphery, were conspicuous (Gore and Isaacson, 1949). Similar changes, with additional cholestasis, were observed by Bragdon (1947) in a patient who died 14 days after fever therapy.

Liver damage has long been recognized as a feature of heatstroke and has been studied in more recent years in percutaneous needle biopsy specimens as well as in necropsy material (Kew *et al*, 1970; Bianchi *et al*, 1972). Mildly damaged livers showed dilatation of central veins and the adjacent sinusoids together with degenerative changes in centrilobular hepatocytes. In more severely affected cases the features were much the same as after fatal heat therapy. In patients who recovered, complete regeneration of parenchymal cells without any residual fibrosis usually occurred within a month (Kew *et al*, 1970; Bianchi *et al*, 1972), cholestasis persisting somewhat longer and a few pigment-laden Kupffer cells still being present for up to a year (Bianchi *et al*, 1972).

Although there have been no previous fine structural studies of human liver after hyperthermia, many of the alterations described here are similar to those produced experimentally in rats (David *et al*, 1971). After exposure to a temperature of  $41^\circ\text{C}$  for 80 minutes increased numbers of autophagic vacuoles were apparent, together with mitochondrial swelling and focal dilatation of the endoplasmic reticulum with ribosomal detachment. One hour later vesicular transformation of the granular endoplasmic reticulum predominated and the cytoplasmic matrix was oedematous. The changes diminished after 4 hours and resolved within 48 hours (David *et al*, 1971).

None of the patients studied here had known liver



metastases, but all were suffering from advanced malignancy which may have affected their livers in some way. Examination of the two available pre-hyperthermia specimens did indeed reveal minor abnormalities and permitted a more accurate evaluation of the changes present after heating, particularly at the fine structural level. Although there were no 'anaesthetic only' controls from our subjects it seems unlikely that the agents used here were responsible for the alterations observed. The livers of control rats after anaesthesia were morphologically normal (David *et al*, 1971) and the extensive morphological changes after hyperthermia reported by earlier workers all occurred in non-anaesthetized subjects.

The liver specimens studied presented a characteristic picture even though the individual changes were themselves nonspecific. Autophagocytosis, the most constant feature in our patients (figs 4 and 5), is a common response to many sublethal agents and is an effective means of disposal of damaged cytoplasmic organelles without the death of the cell (see review by Ericsson (1969)). Sequential post-hyperthermia specimens from case 1 (figs 4 and 5) indicated a rate of formation and turnover of autophagic vacuoles comparable to that in experimental animals after a single stimulus (Glinsmann and Ericsson, 1966). The mitochondrial changes which occurred (figs 6 and 9) are likewise a frequent response to injury (Steiner *et al*, 1964). Dilatation of the rough-surfaced endoplasmic reticulum was observed in all the post-hyperthermia specimens, but particularly in case 3 (figs 7 and 8), where it could be correlated with the clear areas within swollen cells seen after H and E (fig 2) and the purplish patches in toluidine blue stained sections (fig 3). This dilatation differed from that occurring in viral hepatitis (see review by Steiner *et al* (1965)), in that after hyperthermia it often involved only a few cisternae in any one cell (fig 7). The decreased levels of plasma prothrombin and fibrinogen found after hyperthermia (Wilson and Doan, 1940) and heatstroke (Shibolet *et al*, 1967) support the view that this abnormality of the endoplasmic reticulum, as in viral hepatitis, is evidence of defective protein synthesis and secretion.

Pale cytoplasmic inclusions have been reported in other cases of hyperthermia (Bragdon, 1947; Gore and Isaacson, 1949; Bianchi *et al*, 1972) and those present in our specimens (figs 2 and 3) correspond to the large vacuoles seen by electron microscopy (figs 6, 7, and 10). Similar structures in a human hepatoma were considered to be a peculiar type of autophagic vacuole (Enat *et al*, 1973). The particulate material within the vacuoles (figs 6, 7, and 10) presumably represents indigestible cytoplasmic remnants.

Nuclear inclusions, briefly mentioned by Bragdon (1947) and Gore and Isaacson (1949), could be resolved here into three types. Intranuclear lipid and pseudoinclusions of cytoplasmic material are non-specific features of a number of disease states (see Wills, 1968). The flocculent inclusions may have originated as cytoplasmic invaginations since occasionally part of the limiting membrane resembled nuclear envelope (fig 6).

The mechanism of hyperthermia-induced liver injury is uncertain. Circulatory collapse has been implicated (Chunn and Kirkpatrick, 1937) but clearly did not explain all cases. The light microscopic evidence of parenchymal cell vacuolation suggested anoxia as a factor to Gore and Isaacson (1949), but anoxic vacuoles, which are an exaggerated form of normal liver cell pinocytosis (Oudéa and Oudéa, 1967) differ markedly at the fine structural level from those induced by hyperthermia (figs 6, 7, and 10). A more likely explanation appears to be a direct action by the high temperature on the liver cells (Wilbur and Stevens, 1937; King *et al*, 1943; MacDonald, 1944; Gore and Isaacson, 1949; Shibolet *et al*, 1967).

Functional abnormalities cannot always be explained entirely on morphological evidence, and our observations do not account for the altered BSP metabolism in hyperthermia (Findlay *et al*, 1975). Although the canalicular alterations in case 3 (fig 10) indicate an abnormal excretory mechanism, the changes seen can occur secondary to intracellular dysfunction as well as primarily (Biava, 1964; Steiner *et al*, 1965). Furthermore, uptake at the sinusoidal plasma membrane or intracellular metabolism in the cytoplasmic matrix may have been altered without any structural change being obvious.

The degree of liver cell damage seen in case 3 is probably related to the duration of hyperthermia and the maximum temperature attained in this patient. However, the findings in the other two cases indicate that transient liver damage probably occurs in all patients subjected to hyperthermia. This fact should be borne in mind in the assessment of patients for this form of therapy.

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