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Fat necrosis presenting as obscure abdominal mass: Birefringent saponified fatty acid crystalloids as a clue to diagnosis

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Abstract

***Aims*—To describe the birefringent saponified fatty acid crystalloids seen in pancreatic fat necrosis.**

***Methods*—A histological review, including polarising microscopy, of three cases of subacute or subclinical acute pancreatitis was performed. Histochemical analysis using Nile blue sulphate for lipid, Holczinger's copper rubeanate for fatty acids, and Alizarin Red S for calcium was performed in one case. Scanning electron microscopy and *x*-ray energy dispersive spectroscopic microanalysis were performed in two cases. Necropsy pancreatic tissue, surgical archival tissue from cases of pancreatitis, and pancreatic and adipose tissue permitted to autolyse together in the laboratory, were also examined. The autolysed tissue was also examined histochemically. Stained and unstained sections were mounted in DPX and Canada balsam. Surgical material showing traumatic fat necrosis was reviewed.**

***Results*—In each of the three cases there were subtle clues to subclinical pancreatitis. In neither surgical case was the true nature of the mass apparent to the operator. Histological analysis in all cases showed ghost adipocytes containing numerous polarising crystalloids, as well as some basophilic debris. Microanalysis showed calcium but no other substantial heavy element signals. Histochemical analysis showed a labile, polar, acidic lipid and the crystalloids behaved as calcium salts of free fatty acid. The crystalloids were not seen in archival material mounted in Canada balsam. No crystalloids were seen in traumatic fat necrosis.**

***Conclusions*—Little recognised, strongly birefringent, saponified free fatty acid crystalloids occurring in pancreatic fat necrosis may survive routine processing, and can point to the origin of obscure mesenteric masses related to subclinical pancreatitis.**

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Both acute and chronic pancreatitis are well defined clinical syndromes with characteristic presentations. Focal chronic pancreatitis is well known for its presentation with obstructive symptoms and a mass in the head or body of the pancreas, simulating a neoplasm macroscopically and microscopically. A less well known presentation of subclinical or focal subacute pancreatitis, with a mesenteric mass, is illustrated here. Such masses are composed of enzymically damaged adipose tissue containing saponified free fatty acid crystalloids. These little recognised crystalloids may be seen wherever there is pancreatic fat necrosis and may, on occasion, cause diagnostic difficulties in surgical or necropsy material.

Case reports

CASE 1

A 15 cm mass in the mesentery of the small bowel and transverse colon was biopsied in a 67 year old man. Three weeks earlier he had had an episode of upper abdominal pain lasting several hours.

CASE 2

A 31 year old woman with AIDS was treated for suspected tuberculosis but developed jaundice, abnormal liver function, and raised serum amylase. Drug treatment was temporarily stopped and the biochemical test results returned to normal. Her condition deteriorated and she died. Necropsy showed that she had had cytomegalovirus pneumonitis. Within the root of the transverse mesocolon, and affecting the pancreas, was a large, irregular, craggy hard mass with focal necrosis.

CASE 3

A 43 year old man sustained episodes of abdominal pain, jaundice, and vomiting. An ultrasound scan showed cholelithiasis. He was admitted with an exacerbation. He was very tender in the right upper quadrant and a clinical diagnosis of acute cholecystitis/empyema of the gall bladder was made. Serum amylase was 180 U/l (reference range 100–200 U/l). Laparotomy was performed with the intention of cholecystectomy, and revealed a non-inflamed gall bladder with an adjacent large rubbery mass in the omentum. There were also multiple yellow and white nodules seeding the entire peritoneum. The mass in the omentum was thought, possibly,

to be malignant, and the seedlings to be either malignant or fat necrosis. The diagnosis was unclear at surgery; carcinoma of the head of the pancreas or previous pancreatitis were considered.

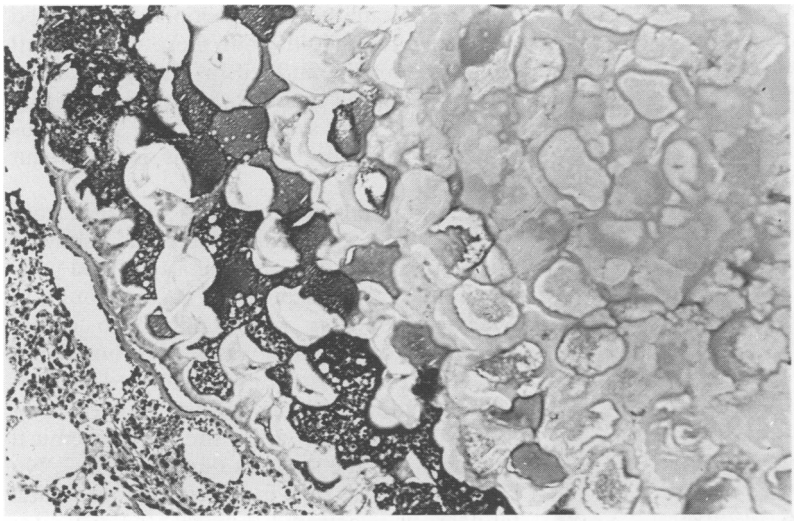
Methods

Haematoxylin and eosin stained sections were available from all cases and paraffin wax embedded material was available from cases 2 and 3. Sections were reviewed by light microscopy and polarising microscopy, with and without a λ plate (first order red compensating filter).

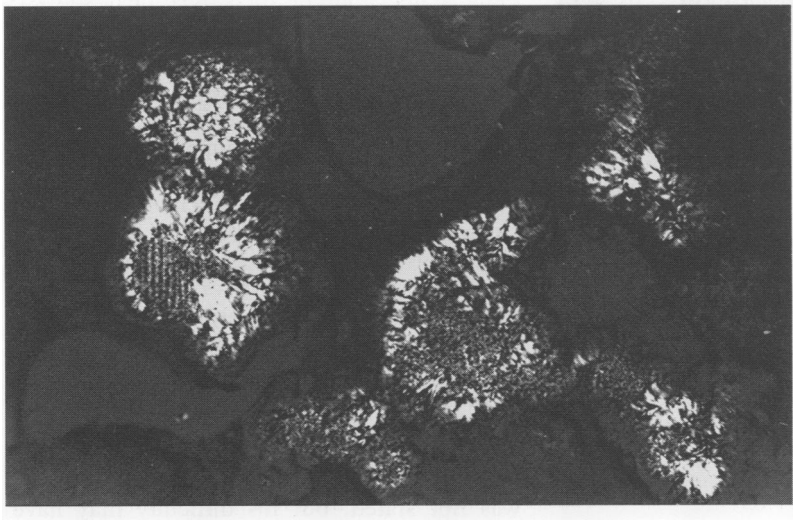
Sections from case 2 were studied by the following techniques to demonstrate lipids: Sudan black, Nile blue sulphate, copper-rubeanic acid and copper-rhodanine. The specificity of these was strictly controlled by using some or all of the following pretreatments: bromination, anhydrous acetone; 70% vol/vol ethanol; molar hydrochloric acid and chloroform methanol (2/1 vol/vol). All lipid methods and treatments were carried out as described before.[1] Calcium was demonstrated by Alizarin Red S.[2]

Paraffin wax sections from cases 2 and 3 were examined by scanning electron microscopy (SEM), using secondary and backscattered electron imaging and *x*-ray energy dispersive spectroscopic microanalysis (EDS). Stained or unstained sections were transferred to a perspex substrate or a carbon stub using an organic based glue. The sections were lightly carbon coated and inserted into the specimen chamber of an Hitachi S520 scanning electron microscope equipped with an S4548 high efficiency scintillator type backscatter detector and a Kevex 4460 δ class analyser. Analysis was performed at an accelerating potential of 15 KV for 100 seconds.

Surgical pathology reports indexed as showing fat necrosis were scrutinised, and in those cases where this was related to pancreatitis the archival sections were reviewed for the presence of polarising crystalloids. Stained and unstained sections recut from the blocks were mounted in DPX and in Canada balsam and examined. The cases reviewed included a biopsy specimen of pancreatitis associated fat necrosis in the subcutaneous tissue over the ankle. Sections from the pancreas and peripancreatic adipose tissue in 10 recent necropsy cases without pancreatitis were reviewed. Non-inflamed pancreatic tissue obtained at necropsy was allowed to autolyse in the laboratory in the presence of adipose tissue, and was thereafter processed, sectioned, and studied histochemically, as already detailed above. Sections of traumatic and postsurgical fat necrosis were reviewed, and in one case recut and an unstained section mounted in DPX.



(A)



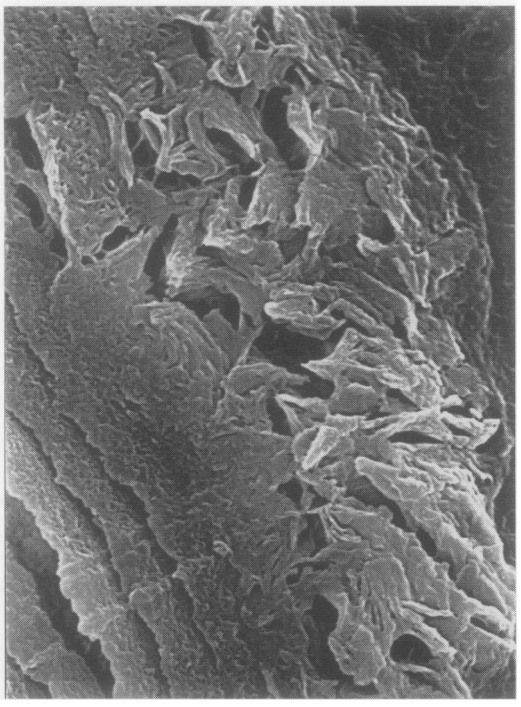
(B)

Figure 1 (A) The edge of a lobule of necrotic fat in case 3. The ghost adipocytes to the right contain polarisable crystalloids (haematoxylin and eosin). (B) Polarising crystalloids in ghost adipocytes in the same lobule (haematoxylin and eosin).

Results

Histological analysis in each of the three cases showed fat necrosis with retention of recognisable adipose tissue architecture in the necrotic areas. There was a variable degree of inflammatory cell infiltrate at the margin of the necrotic process. Each lobule consisted of ghost adipocytes containing numerous polarising crystalloids (fig 1). Insertion of a first order red compensating filter enabled the birefringence of these crystalloids to be characterised as negative, as determined by comparison with urate crystals (negative) and pyrophosphate crystals (positive) in tissue sections. The crystalloids varied greatly in size, the largest being about 12 μm $\times$ 1 μm. Often they were seen in radial sheaves within some of the ghost adipocytes, and crosscut in others. In some lobules the necrotic cells contained basophilic or amphophilic debris centrally, and the crystalloids were arranged peripherally in the cells. The larger crystalloids tended to be located toward the centre of the lobules, with finer crystalloids in the peripheral cells. The extreme manifestation of this variation in size was seen in areas where the crystalloids were no longer discernible, leaving a birefringent haze with a propensity to show a fine

(A)



(B)

Figure 2 (A) Scanning electron micrograph of a paraffin wax section showing ghost adipocytes containing saponified free fatty acid crystalloids. (B) Higher power showing varying size of crystalloids and sectioning artefact.

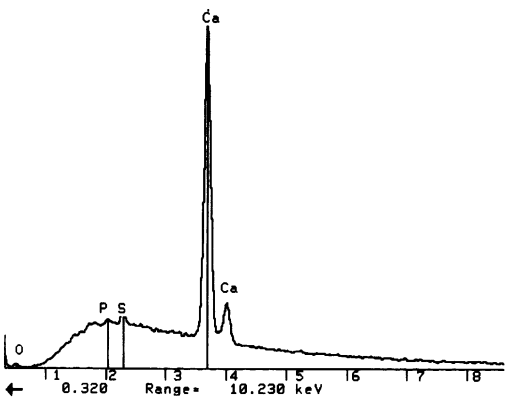


Figure 3 Spectrum from crystalloids on microanalysis.

"chatter" sectioning artefact. Some ghost adipocytes contained yellow, apparently amorphous material; viewed under strong polarised light, most of this was also found to contain birefringent crystalloids.

The crystalloids proved rather labile in the scanning electron microscope. Convincing images (fig 2) were obtained only under optimal conditions with a very light carbon coating and examination at low accelerating voltage. EDS for 100 seconds showed an almost pure calcium signal with no other relevant peaks, although low pulse counts were obtained for oxygen, phosphorus and sulphur (fig 3).

Histochemical analysis using Sudan black failed to show the presence of any lipid material, but Nile blue sulphate showed a polar, acidic lipid. Holczinger's copper-rubeanate and Alizarin Red S confirmed crystalloids behaving as calcium salts of free fatty acid.

Review of the archival sections of biopsied surgical cases of pancreatic fat necrosis showed no polarisable crystalloids. In recut sections, however, polarising crystalloids were present in DPX mounted sections, whether stained or unstained. Unstained Canada balsam mounted sections also showed polarising crystalloids, but these were absent in haematoxylin and eosin stained Canada balsam mounted sections. Identical polarising crystalloids were seen in the case of subcutaneous pancreatic fat necrosis in the recut sections.

Sections from non-inflamed pancreas from recent routine necropsy material showed some crystalloid deposition in saponified peripancreatic fat in eight of 10 cases, though in five of these this was confined to a few adipocytes only. These cases were not recut. The in vitro experimentally autolysed pancreatic and adipose tissue studied showed crystalloid deposition and the histochemical findings were the same as those in case 2. No crystalloids were seen in the cases of traumatic fat necrosis reviewed.

Discussion

Pancreatic fat necrosis presenting as a mass in the root of the transverse mesocolon may cause diagnostic problems. These three illustrative cases should, hopefully, heighten awareness of this possible presentation. In each case the link with pancreatitis was not clear at first. Crystal deposition disease had been considered in the first case and a vegetable or parasitic origin of the birefringent material was originally suspected in the second. In the third case the surgical differential diagnosis included malignancy.

Baggenstoss[3] described basophilic calcium soap formation in pancreatic fat necrosis. Unlike us he had difficulty demonstrating the calcium histochemically. His methodology was not stated, but his difficulty may have been related to the absence of phosphate. He did, however, mention the occasional presence of fatty acid crystals. Gyr *et al*[4] produced a detailed account of the morphological

changes in pancreatic fat necrosis. They described saponified basophilic calcium precipitates occurring as a result of release of fatty acids by hydrolysis. They did not mention crystals in their report.

The crystalloids in our cases did not stain with Sudan black even after prior bromination. Sudan black is regarded as an efficient overall means of screening for all classes of lipids. Some lipids, in particular free fatty acids, may be soluble in the 70% ethanol of the dye bath and may not be retained in the tissues. In addition, only those lipids which are liquid at staining temperature take up the dye. Free fatty acids may be stabilised in tissues by sequestering calcium ions to form soaps, either in vivo or in a calcium containing fixative.[5] Their full histochemical reactivity (and solubility) can be restored by de-saponifying with hydrochloric acid. After de-saponification, Sudan black failed to stain the crystalloids, but Nile blue sulphate stained these as free fatty acids. It seemed that the crystalloids were soluble in 70% ethanol (the solvent for Sudan black). We later confirmed that 15 minutes or less in 70% ethanol is sufficient to remove the crystalloids from sections, as evidenced by loss of birefringence and Nile blue sulphate staining. Unlike most other "fat" stains, Nile blue sulphate is a completely aqueous solution and comprises two components. The red component is organotropic (sudanophilic) and stains neutral non-polar lipids (triglycerides) red; the blue component binds in a charged manner to polar lipids (free fatty acids and phospholipids).

It is unclear why archival material fails to show polarisable crystals. It could possibly be the effect of the storage time, or could be related to the use of mountants with differing optical properties, or to the tendency to become acid on storage. Perhaps a similar explanation could be that a slight variation in the standard time for haematoxylin and eosin staining with a slightly prolonged exposure to 70% ethanol on rehydration is sufficient to destroy them. Additionally, some haematoxylin formulations do, of course, contain fairly large amounts of ethanol—for example, Ehrlich's haematoxylin. This could explain why these crystalloids have been largely unrecognised in the past. The phenomenon of crystalloid deposition in fat necrosis may be observed wherever pancreatic enzymes are able to act on adipose tissue. Such situations include pancreatitis, whether subclinical or subacute, as in these cases, or in typical acute cases, and it may involve mesenteric adipose tissue, or, rarely, subcutaneous fat. In theory the same process may occur after duodenal perforation. This phenomenon also occurs in relation to the non-inflamed pancreas as a post mortem autolytic process, in a similar way to that demonstrated after permitting pancreatic and adipose tissues to autolyse in contact at room temperature in the laboratory.

These crystalloids survive routine processing but may or may not be polarisable, depending on the mountant used. Polarisation of mounted unstained sections generally confirms their presence. The presence of such crystalloids may give a clue to the origin of obscure mesenteric masses related to subclinical pancreatitis.

We thank Dr M Stephens and Dr JG Winnick for contributing cases 1 and 2.

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