THE SPALLING AND MIGRATION OF SILICONE FROM BLOOD PUMP TUBINGS IN HAEMODIALYSIS PATIENTS

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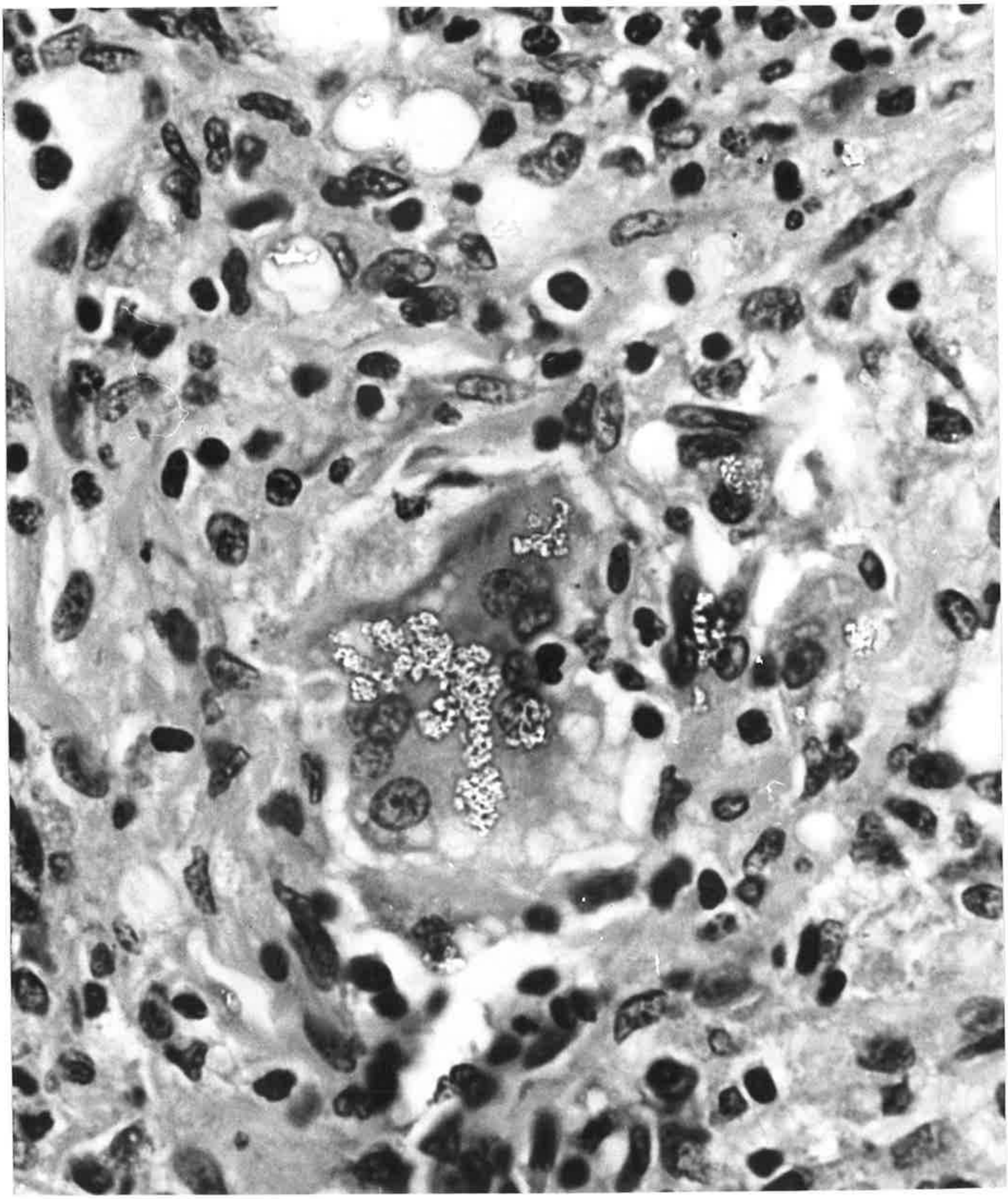
TYPICAL SILICONE GRANULOMA



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SUMMARY

This study was instituted following the observation of an unstainable, refractile, non-birefringent material in liver biopsy specimens from renal patients. Retrospective examination of tissues from selected clinical populations and suitable controls revealed that the material was present only in patients treated with repeated haemodialysis. Of 38 haemodialysed patients who had liver biopsies, 18 showed varying amounts of the material. Of 31 haemodialysed patients who came to autopsy, 22 showed accumulations of the contaminant. The substance had embolised in the blood and was engulfed by macrophages and stored in the mononuclear phagocytic system. Disseminated granulomata were present in the liver, spleen and lymph nodes. The ultrastructural appearances were characteristic and very suggestive of a plastic-like substance. The particles were found to be located in macrophage lysosomes. Filings from new silicone tubings from the roller pump segment of the haemodialysis machine introduced subcutaneously into rats produced a granulomatous response with particulate inclusions of a similar light and electron microscopic appearance.

Local and overseas consultation did not help identify the material. After initial setbacks the contaminant was confirmed to be silicone by electron dispersive X-ray analysis and back-scattered electron imaging. The analysis of silicone in small quantities is fraught with problems. Atomic absorption spectrometry allowed quantitation of silicone in several autopsy tissue samples. Detectable quantities were found in the liver, spleen, lymph node and other organs. The accumulation of hepatic silicone correlated with the duration of exposure. A positive correlation could also be demonstrated between the amount of silicone and the severity of hepatic fibrosis and inflammation. Seventeen biopsy and 10 autopsy cases with hepatic

deposits of silicone showed raised levels of serum aspartic transaminase (AST), and eight autopsy cases had normal levels of this enzyme. Eleven patients showed elevations of serum AST which exceeded a six-month duration (chronic hepatitis). After consideration and exclusion of other known causes of chronic hepatitis, it was felt that silicone-induced hepatitis was a possible cause of chronic liver dysfunction in the haemodialysed patient.

Silicone or polydimethylsiloxane is widely employed in medicine, pharmacy and cosmetics. Despite its stability and relative physiological innertness there is ample experimental and clinical evidence to indicate that it can induce a chronic inflammatory response. Subcutaneous injections of fluid silicone in the rat evoked an initial inflammation which lasted up to six months. Subsequent examination revealed fibrous encapsulation of the deposit with pericapsular macrophages, lymphocytes and giant cells. Massive localised doses of silicone produced striking abnormalities of adipose tissue throughout the body and the material was dispersed in many organs. The fibrogenic and inflammatory properties of silicone have also been seen in clinical situations. Silicone mastitis is a chronic granulomatous reaction around deposits of silicone fluid, gel or elastomer introduced for breast augmentation. Migration of the material produces a granulomatous inflammation in draining lymph nodes. Illicit injections of silicone have produced disseminated granulomata. In such cases elevated levels of serum hepatic enzymes have been recorded in association with granulomata in the liver. Reports of fatal and near-fatal allergic-type responses to silicone elastomer prostheses and subcutaneous injections have described a febrile systemic illness associated with diffuse arthritis, renal failure and an adult respiratory distress syndrome. Other examples of clinical migration of silicone with production of disseminated granulomata include the decay and fragmentation of silicone balls in ball-valve cardiac prostheses. The migration of silicone from orthopaedic prostheses has also produced granulomata in the adjacent synovium and draining lymph nodes. In all these instances refractile particles were observed in phagocytic cells.

The presence of silicone in the liver of our patients was consistently associated with fibrosis whereas inflammation was variable. Experimental studies indicate that silicone initiates an inflammatory response which subsides as fibrosis supervenes and isolates the foreign material. It is suggested that this phasic reaction pattern might be reflected as variations in serum AST levels.

While there is strong evidence to show that silicone might be a primary aetiologic factor in the causation of chronic hepatitis, it is possible that silicone may also function in a synergistic manner to prolong hepatic inflammation and dysfunction initiated by other causes. In the presence of depressed erythropoiesis and impaired immune function which commonly accompanies chronic renal failure, it was not possible to fully evaluate any functional effects that silicone deposition may have had in the bone marrow, spleen and lymph nodes.

Finally, the source of silicone spallation was proven by in vitro testing of new silicone tubings. Fragmentation of the inner walls of the tubing was demonstrated by scanning electron microscopy and atomic absorption spectrometry of the effluent blood. It is strongly recommended that this source of contamination in extracorporeal circulation be immediately replaced.

CONTRIBUTIONS OF THIS THESIS

This dissertation makes the following contributions:

1) it identifies the silicone roller pump tubing as a source of particulate contamination in extracorporeal circulation of blood;

2) evidence is presented to show that silicone deposition in the liver is a previously unrecognised potential cause of chronic hepatitis in haemodialysis patients;

3) the migration, storage and tissue reactions to this foreign material are described;

4) the morphological features of silicone in body tissues are characterised;

5) the diagnostic approach to a new morphological observation is outlined and the use of various modern optical, ultrastructural, and analytical techniques which can be employed to the identification of histochemically non-reactive particles in tissue sections is demonstrated;

6) it demonstrates the application of the relatively new technique of electron dispersive X-ray analysis for the identification of microscopic quantities of non-biological elements and confirms the enhancement of this technique when it is combined with back-scattered electron imaging;

7) the difficulties involved in the analysis of silicone are reviewed and atomic absorption spectrometry is established as a useful method in the detection and quantitation of small amounts of silicone in wet tissues and blood;

8) it describes a method of objective quantitation of the morphologic variables seen in tissue sections in association with the presence of silicone;

9) the medical applications of silicone, its clinical complications and potential hazards are reviewed;

10) spallation studies of silicone tubings are performed and scanning electron microscopy and atomic absorption spectrometry confirm the source of silicone emboli;

11) the causes and complications of particulate contamination in haemodialysis are reviewed;

12) it shows the results of experimental introduction of silicone filings into the subcutaneous tissue of rats;

13) it describes the light microscopic and fine structural changes associated with silicone mastitis in humans.

ACKNOWLEDGEMENTS

I am grateful to my supervisor, Professor A G Wangel, for his patient and helpful criticisms of this dissertation. My sincere thanks to Mr David W Gove, my research associate, who spent many hours assisting with the technical aspects of this investigation and also to the staff of the electron microscopy suite at the Queen Elizabeth Hospital for their cheerful and willing help. Dr A P S Disney made available the clinical data in this study. Mr J Waters and Mr P Schultz of AMDEL analysed the tissue and blood samples for silicone.

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I. INTRODUCTION

I. INTRODUCTION

Over two years ago during routine diagnostic microscopic examination, the author first noticed the presence of refractile particles in liver biopsies accessioned at the Histopathology Department of the Queen Elizabeth Hospital, Woodville. This thesis describes the investigations which were undertaken to characterise this foreign material and relates the events which subsequently led to the identification of a previously unrecognised iatrogenic disorder found in patients treated by repeated haemodialysis.

In an attempt to preserve the deductive sequences, this dissertation follows the chronological order in which the investigation was conducted. This somewhat unconventional mode of presentation will also provide a more accurate reflection of some of the problems encountered in the study, many of which resulted from difficulties in obtaining expert advice on analytical facilities.

II. MORPHOLOGICAL CHARACTERISTICS OF A FOREIGN MATERIAL IN THE LIVER

II.a. LIGHT MICROSCOPY

II.b. POLARISATION MICROSCOPY

II.c. PHASE CONTRAST MICROSCOPY

II.d. ELECTRON DIFFRACTION STUDIES

II.e. ELECTRON MICROSCOPY

II.f. SUMMARY

II. MORPHOLOGICAL CHARACTERISTICS OF A FOREIGN MATERIAL IN THE LIVER

II.a. LIGHT MICROSCOPY

The foreign material observed in routinely prepared liver biopsies occurred in varying quantities as small particles or as larger clumps. It displayed irregular and seemingly crenated or crinkled outlines. It was colourless and transparent and because of its prominent refractile properties appeared to be inorganic in character. Its refractility was greatly enhanced by flipping out the microscope condenser unit and closing the substage diaphragm to a minimum (Figure 1a). It did not stain with common histochemical techniques. Periodic acid-Schiff, Sudan black, Oil red O, alkaline silver, Schmorl and acid fast stains were all negative. Additional stains for iron and calcium were also negative and the material did not show autofluorescence when viewed in ultra-violet light.

II.b. POLARISATION MICROSCOPY

Polarisation microscopy is a useful method in the identification of foreign material in tissue sections. The properties of dichroism and birefringence are found in anisotropic or optically unhomogeneous objects whose molecules or other submicroscopic units are arranged in a definite orientation rather than a random arrangement (Pearce, 1972). The refractile particles,however, displayed neither dichroism or birefringence when viewed in polarised light, i.e. the material was isotropic. Because most crystalline substances tend to possess either of these properties, their absence suggested that the material observed in the liver biopsies was not crystalline in nature.

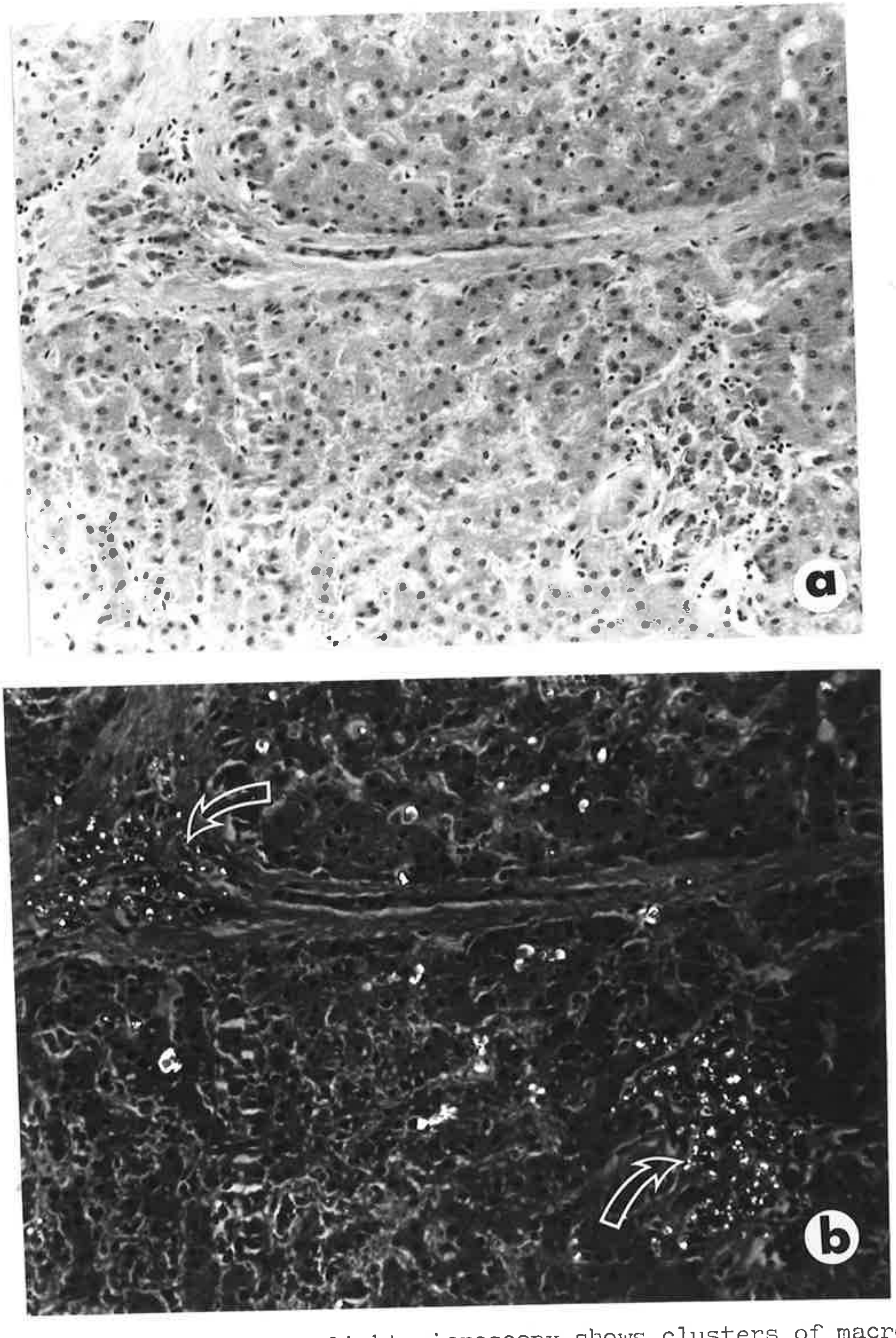

Figure 1: (a) ordinary light microscopy shows clusters of macrophages in expanded portal areas of the liver; (b) same field viewed by phase contrast microscopy. Brightly contrast negative particles are clearly present in the macrophages (arrows Some erythrocytes in the sinusoids are also contrast negative (H & E, x 200).

II.c. PHASE CONTRAST MICROSCOPY

The phase contrast microscope renders transparent objects visible by converting phase changes in the light passing through the object into changes of wave amplitude. For this to occur the object of study should have a refractive index which is different from that of the embedding medium. The difference is reflected as regions of differing brightness in the tissue section. It therefore seemed appropriate to examine the refractile particles by phase contrast microscopy. The particles were found to be brightly contrast negative (Figure 1b) and the technique proved to be a useful means of confirming and detecting the presence of the material especially when it occurred in small quantities. However, aside from enhancing the accuracy of detection, this method of study contributed no further information towards identifying the substance.

II.d. ELECTRON DIFFRACTION STUDIES

Electron or X-ray diffraction studies have been demonstrated to be useful in determining the chemical and crystalline nature of mineral inclusions (Berry et al, 1976). Concentric diffraction rings indicate the crystalline nature of the object and specific metals produce characteristic patterns which allow their identification. Three biopsies with the refractile material were studied by electron diffraction and the findings confirmed the polariscopy observation that the material was not of a crystalline nature.

II.e. ELECTRON MICROSCOPY

The most striking ultrastructural feature of the refractile particles was their strong resemblance to the plastic embedding medium used in the electron microscopic preparation. The material had generally rounded outlines and was of varying sizes. It was uniformly electron lucent and showed a characteristic tendency to "burn" when examined in the electron beam in a manner not dissimilar to that seen with the embedding medium. With prolonged bombardment by electrons the material continued to "burn" and to curl up around the edges of the empty space left by the "burn". This produced a rim of electron density around the "burn" hole and, together with the occasional residual strand of the material which bridged the hole, produced a characteristic appearance which was readily recognised even at low magnifications (Figure 2). The latter features differed from the behaviour of embedding media such as Spurr and Epon which "burn" as enlarging rounded defects or holes with stretching edges of uniform electron lucency.

II.f. SUMMARY

At this stage of the investigation it appeared that the refractile foreign material observed in routinely prepared liver biopsies was not likely to be crystalline in nature and it showed a strong ultrastructural resemblance to plastic.

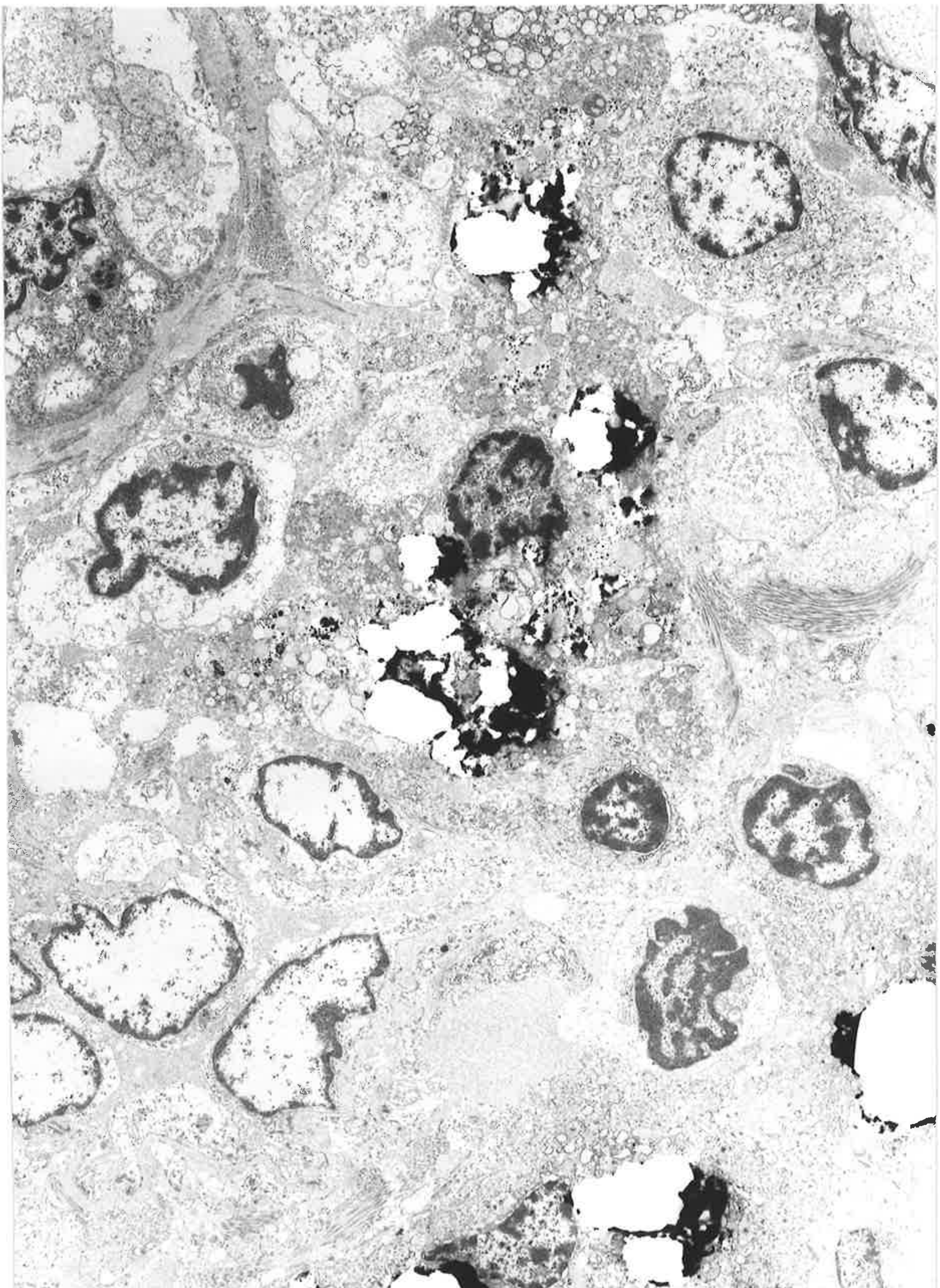

Figure 2: Electron micrograph of hepatic portal tract showing characteristic inclusions in macrophages (Autopsy Case 17, uranyl acetate-lead citrate, X 5,000).

III. MATERIAL AND METHODS

III.a. MATERIAL EXAMINED

Antemortem Material

Postmortem Material

Controls

III.b. METHODS

III. MATERIAL AND METHODS

III.a. MATERIAL EXAMINED

The author's previous experience with liver tissues (Leong, 1976; Leong & Sage, 1977; Leong, 1978; 1980; Leong & Alp, 1981) indicated that the refractile particles were not an artefact of processing. As the morphologic characteristics gave little indication of the nature of the particles, it was necessary to examine tissues from selected clinical populations for possible clues to their origin. It rapidly became apparent that the material was encountered only in liver biopsies from patients treated by the Renal Unit of the hospital. It also appeared that only patients with chronic renal failure treated with repeated haemodialysis accumulated the foreign material in their tissues.

The material studied retrospectively consisted of antemortem biopsies and postmortem tissues. These were separated into cases with chronic renal failure treated by haemodialysis and those who were treated by other means. In addition, suitable controls from patients not treated by the Renal Unit were examined (Table I).

Ante mortem Material

All antemortem liver biopsies from patients treated by haemodialysis at the Queen Elizabeth Hospital were culled from the files of the Histopathology Department. Only biopsies obtained between 1973 and 1980 were examined as departmental records before 1973 were inaccurate and slides and tissues were frequently unavailable for examination. Between January 1973 and June 1980 a total of 42 biopsies from 38 patients were available for study. Among these patients was a group of 15 cases who were serologically positive for hepatitis B surface antigen (HBsAg) and had been biopsied as part of a viral hepatitis study (Coughlin et al, 1980). The other patients had liver biopsies because of clinical or biochemical liver dysfunction.

Table I: MATERIAL EXAMINED

LIGHT MICROSCOPY

Biopsy - 38 patients
(15 patients HBsAg positive)

Autopsy - 31 patients
(1 patient HBsAg positive)

Controls - 10 patients with chronic renal failure
(6 treated with peritoneal dialysis only,
4 not dialysed at all)

- 80 randomly selected biopsies from non-renal patients

- 50 consecutive autopsies of non-renal patients

ELECTRON MICROSCOPY

10 autopsy liver samples and 2 spleen samples known to contain the refractile contaminant

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All but one were closed liver biopsies.

Postmortem Material

Between 1976 to 1980, a total of 31 patients who had been treated by haemodialysis came to autopsy. All available tissues from these cases were examined retrospectively.

Controls

Liver biopsy tissues from two patients who had renal failure but who had not been treated by haemodialysis were available for examination. As many as 80 liver biopsies randomly selected from patients who had no renal complaints served as controls.

Postmortem tissues from 10 patients with chronic renal failure who had not been haemodialysed were examined as controls. Six of these cases were treated by peritoneal dialysis only and no dialysis procedure at all was done in the others. Material from a further 50 consecutive autopsies of patients who were not in renal failure were selected as controls.

III.b. METHODS

All biopsies and postmortem tissues had been fixed in 10% formalin and embedded in paraffin. They were cut at 4-8 microns in thickness and were routinely stained with haematoxylin and eosin for examination by light microscopy. In addition, Masson's trichrome, Periodic acid-Schiff with and without diastase digestion, reticulin, elastic van Gieson, bile and Perl's stains were performed when appropriate. All haematoxylin and eosin-stained sections were further examined by phase contrast microscopy for the presence of the refractile particles. In the event that preservation was poor or slides were missing, new sections were cut from paraffin blocks in storage. In all autopsies but one, postmortem examination was complete and tissues of most organs were available for study.

In 10 autopsies wet formalin-fixed liver tissue was available. Preparation for electron microscopic examination was carried out in a standard manner (Leong, Chawla & Teh, 1978; Leong et al, 1980; Leong, 1982). Small portions of liver were diced into 0.5-mm cubes and transferred into a 0.1 M sodium cacodylate buffered 1% glutaraldehyde-4% formaldehyde mixture (McDowell & Trump, 1976) and post-fixed in osmium tetroxide. The tissue was subsequently dehydrated in alcohol and embedded in Epon or Spurr. One micron-thick sections stained with toluidine blue were examined for selection of suitable areas to study and thin sections stained with uranyl acetate and lead citrate were examined in an AEI type 801 electron microscope. In addition to liver samples, two portions of spleen were similarly prepared for examination by electron microscopy.

IV. <u>DISTRIBUTION AND TISSUE REACTIONS TO THE FOREIGN MATERIAL</u>

IV.a. LIGHT MICROSCOPIC OBSERVATIONS

Liver Pathology
Splenic Pathology
Lymph Node Pathology
Bone Marrow Pathology
Pulmonary Pathology
Other Tissues

IV.b. ELECTRON MICROSCOPIC OBSERVATIONS

Ultrastructural Observations in the LIver
Ultrastructural Observations in the Spleen

IV. DISTRIBUTION AND TISSUE REACTIONS TO THE FOREIGN MATERIAL

IV.a. LIGHT MICROSCOPIC OBSERVATIONS

The refractile particles were found only in tissues of patients who had been treated with repeated haemodialysis. All liver biopsy and autopsy specimens fron "non-renal" patients which served as controls showed no evidence of the refractile particles even by phase contrast microscopy. Material from patients with chronic renal failure who had not been treated by haemodialysis or who had received only peritoneal dialysis did not contain the particles.

Of the 38 patients with chronic haemodialysis who had had liver biopsies, 18 showed varying amounts of the material. Four of these patients were positive for HBsAg. Of the 31 autopsies, 22 patients revealed the presence of the refractile material in their livers, 5 of these having had previous liver biopsies (Table II).

Liver Pathology

By light microscopy, the particles were isotropic and refractile. They were transparent and colourless and were best demonstrated by phase contrast microscopy which rendered them brightly contrast negative. The material was present in varying quantities in the liver ranging from barely visible particles within macrophages (Figure 1a) to large clumped aggregates in multinucleated giant cells (Figure 3) or as free particles in the stroma of expanded portal tracts. Less frequently they were seen in the lobules and around central veins. Kupffer cells lining sinusoids occasionally contained the contaminant (Figure 4) and in one case (Autopsy Case 22) large flakes of the material were observed distending congested sinusoids (Figure 5). The presence of these particles was associated with variable degrees of inflammation and fibrosis in the liver (Leong, 1981).

Table II. DISTRIBUTION OF REFRACTILE PARTICLES IN AUTOPSY AND BIOPSY MATERIAL EXAMINED BY LIGHT MICROSCOPY

Tissue Examined	Number of Positive Cases
AUTOPSY MATERIAL (total number examined 31)	
liver	22
Cases with hepatic granulomata	9
spleen	22
lung	8
bone marrow	9
lymph node	2
BIOPSY MATERIAL (total number examined 38)	
liver	18 (4 HBsAg +ve)

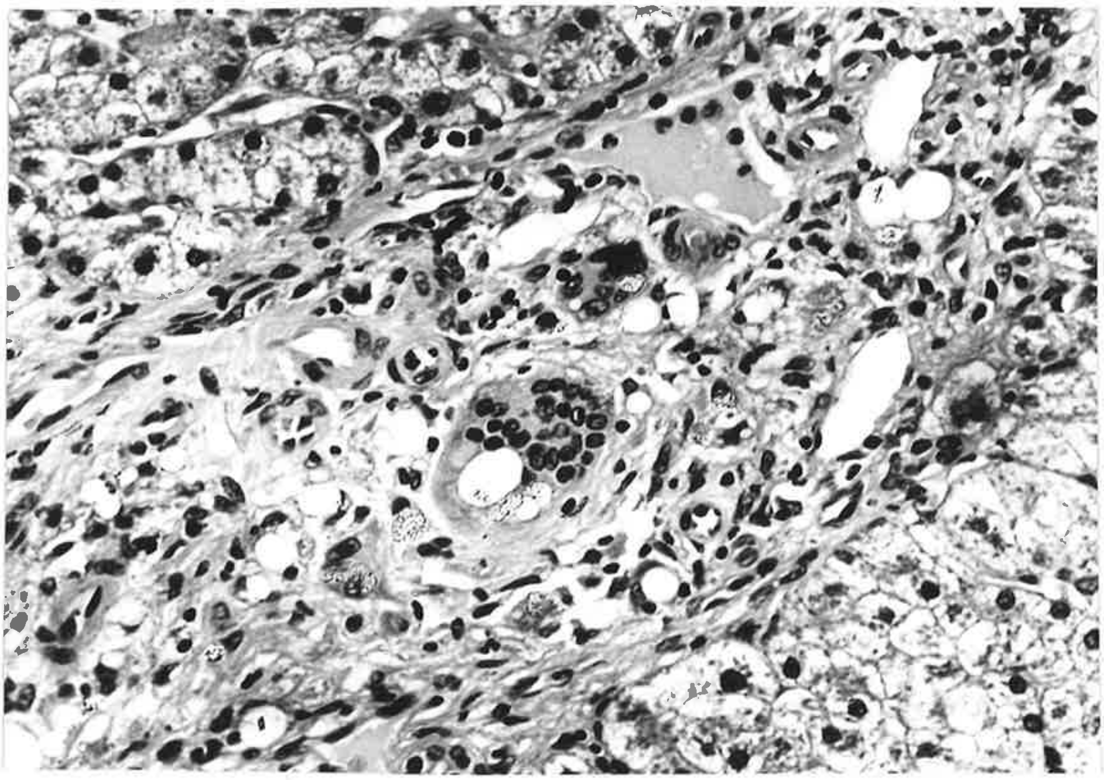


Figure 3: Portal tract granuloma with giant cells containing clumps of refractile material (H & E, x 550)

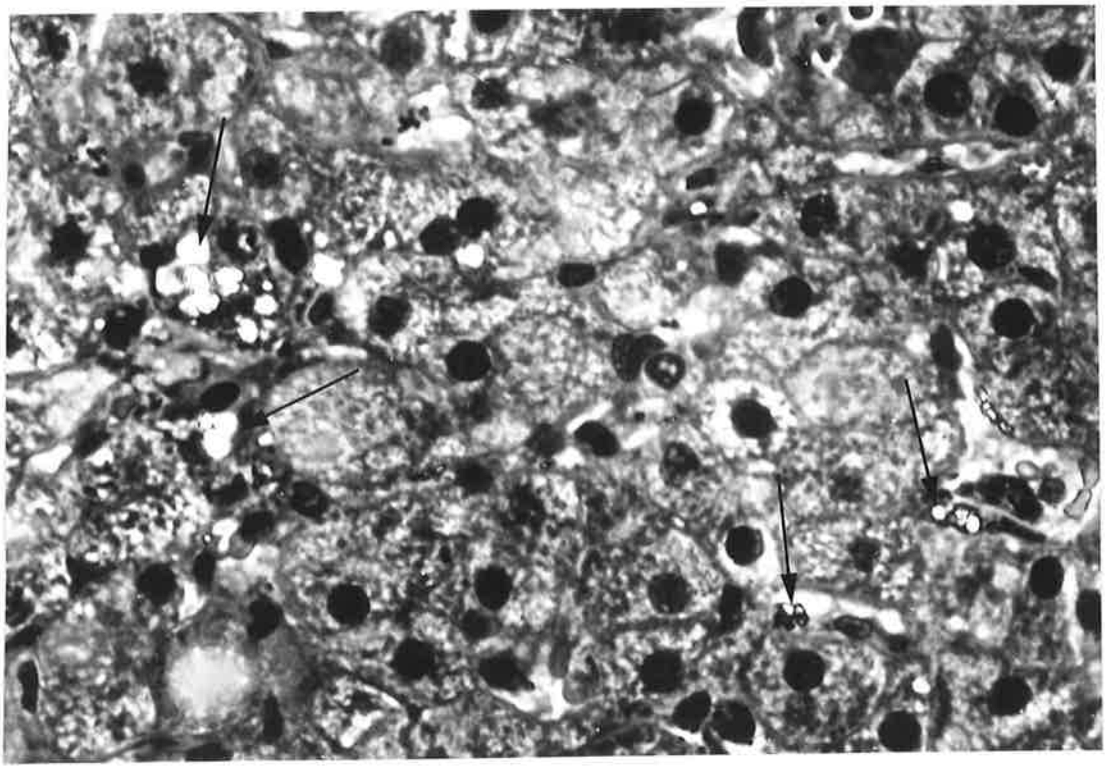


Figure 4: Refractile particles are seen in Kupffer cells lining hepatic sinusoids (arrows) (H&E, x 850).

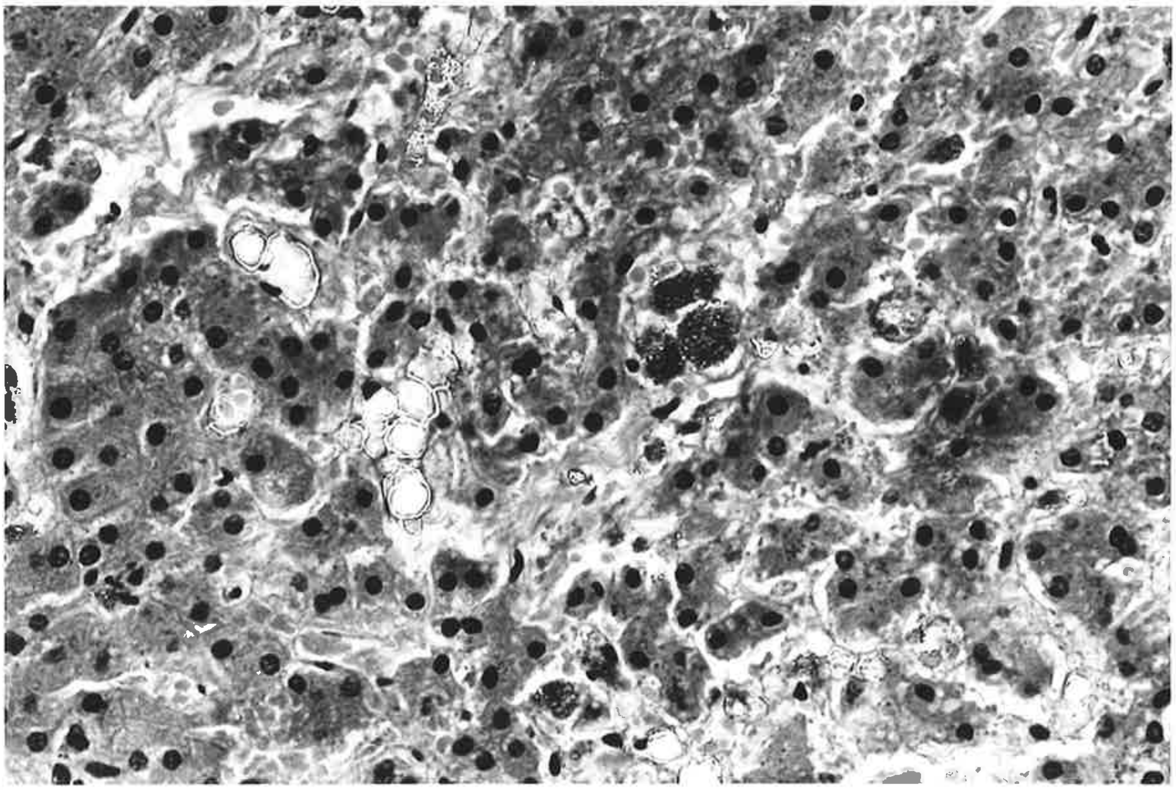


Figure 5: Large flakes of refractile material are seen distending hepatic sinusoids. Kupffer cells contain haemosiderin pigment and some granular refractile particles (Autopsy Case 22, H & E, x 550).

A quantitative method of assessing the amount of refractile material, the severity of inflammation and the extent of fibrosis in the liver sections was devised. These estimations were made on a coded basis with no knowledge of the clinical data. The amount of refractile material in the liver sections was quantitated on a scale of 0 to 4, where 0 = absent, 1 = barely visible particles, 2 = readily visible particles, 3 = clumped particles and 4 = confluent clumps or abundant particles (Figure 6). The degree of inflammation and extent of fibrosis associated with these particles were assigned scores in the following manner: for inflammation, 0 = absent, 1 = mild inflammation, 2 = moderate inflammation without giant cells or granuloma formation, 3 = moderate inflammation with giant cells or granuloma formation and 4 = severe inflammation with or without granuloma formation (Figure 7). For fibrosis, 0 = absent, 1 = expansion of portal tracts by fibrosis, 2 = stellate scars in portal tracts, 3 = linkage of stellate scars in adjacent portal areas and 4 = encircling of nodules of hepatocytes by fibrous scars (Figure 8). The results of this quantitation are presented in Tables IIIa and IIIb. Quantitation was similarly performed on biopsy and autopsy liver sections which did not contain the refractile material and these results are presented in Tables IIIc and IIId.

The hepatic inflammation seen in association with the refractile particles tended to be located in portal tracts and consisted of infiltrating lymphocytes, macrophages, immunoblasts and plasma cells. It was generally mild to moderate in severity with a few instances where there was virtually no inflammatory response to the presence of the material. In three biopsy cases and in nine autopsy liver samples a granulomatous inflammation of multinucleated giant cells and loose aggregates of epithelioid histiocytes was seen. In all instances the refractile particles were located within phagocytes and giant cells (Figure 9) or were extracellularly sited in the fibrous stroma. Although foci of hepatocyte drop-out occurred, significant lobular inflammation was not seen. In only three biopsies was inflammation considered to be of a severe degree (one of these cases subsequently came to autopsy and showed a similar degree of inflammation - Autopsy Case 11). In two biopsies piecemeal necrosis was evident. One of these came from a HBsAg positive patient who also showed typical

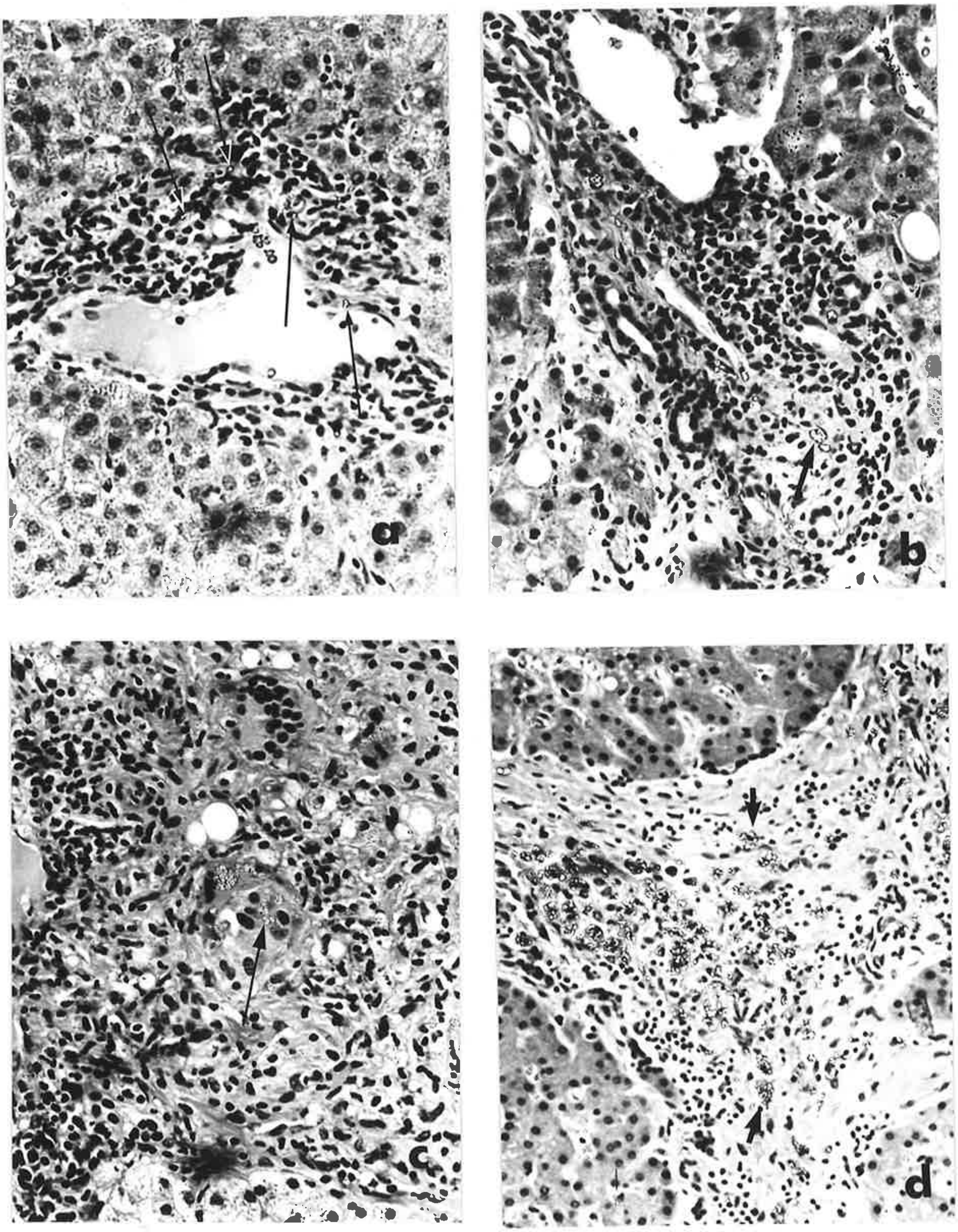


Figure 6: Quantitative scoring for the refractile material (silicone). a) grade 1, barely visible, b) grade 2, readily visible, c) grade 3, clumped partilces, d) grade 4, abundant (H & E, particles are arrowed).

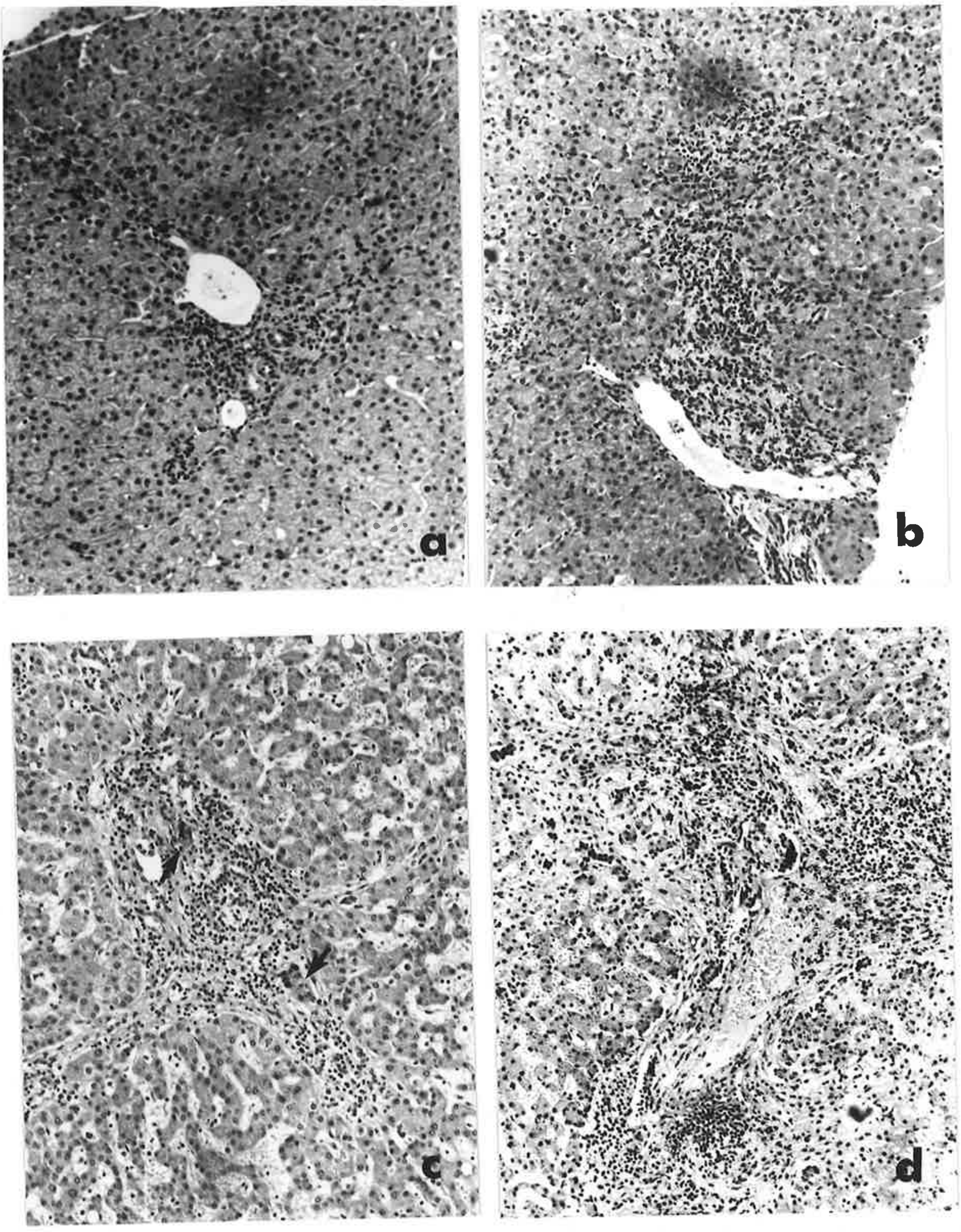


Figure 7: Grades of inflammation associated with refractile particles. a) grade 1, mild inflammation, b) grade 2, moderate inflammation without giant cells or granuloma, c) grade 3, moderate inflammation with giant cells (arrows) and granuloma, d) grade 4, severe inflammation (H & E, x 125).

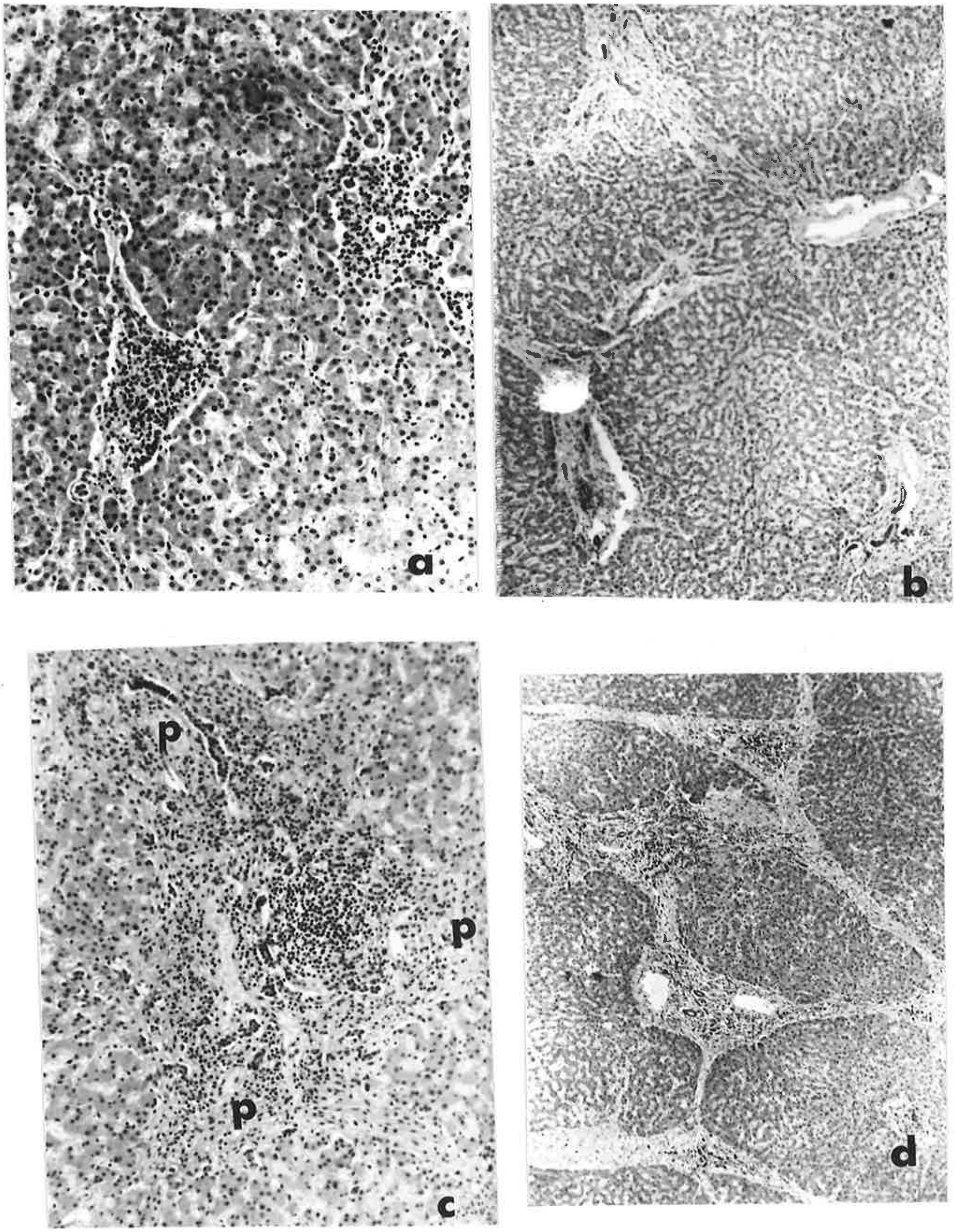


Figure 8: Grades of fibrosis associated with deposition of refractile material in the liver. a) grade 1, expansion of portal tracts by fibrosis, b) grade 2, stellate scars, c) linkage of scars between portal areas (P), d) encircling of nodules by fibrous bands (H & E).

Table IIIa: Quantitation of Refractile Particles, Fibrosis and Inflammation in Liver Sections: Biopsy Cases

Case No.	Refractile Particles	Fibrosis	Inflammation
1	1	2	1
2+	1	1	1
3	1	2	2
4	2	2	2
5	1	2	2
6+	1	3	4
7+	1	1	1
8	2	1	1
9	3	3	4*
10	2	3	1
11	2	2	1
12	2	1	2
13	2	1	0
14	3	2	3*
15	3	2	1
16+	2	2	2
17	4	3	4*
18	3	3	2

+ = HBsAg positive cases; * = with giant cells or granuloma

refractile particles scoring: 1=barely visible, 2=readily visible, 3=clumped aggregates, 4=confluent clumps
fibrosis grading: 1=expansion of portal tracts by fibrosis, 2=stellate scars in portal areas, 3=linkage of stellate scars, 4=encircling of nodules of hepatocytes by firbous bands
inflammation grading: 0=absent, 1=mild, 2=moderate without giant cells or granuloma, 3=moderate with giant cells or granuloma, 4=severe with or without giant cells or granuloma

Table IIIb: Quantitation of Refractile Particles, Fibrosis and Inflammation in Liver Sections: Autopsy Cases

Case No.	Refractile Particles	Fibrosis	Inflammation
1	1	2	1
2	1	2	2
3	1	2	1
4	3	2	2
5	1	1	0
6	4	2	3*
7	1	1	0
8	1	1	0
9	1	4	2
10	2	3	1
11	3	3	4*
12	3	2	3*
13	3	2	2*
14	3	2	1
15	3	2	3*
16	2	1	3*
17	4	2	3*
18	3	1	3*
19	2	2	1
20	3	4	3*
21+	3	3	2
22	4	4	2

+ = HBsAg positive case
* = granulomas present

Refractile Particles scoring: 1=barely visible, 2=readily visible, 3=clumped particles, 4-confluent clumps.
Fibrosis grading: 1=expansion of portal tracts by fibrosis, 2=stellate scars, 3=linkage of stellate scars, 4=encircling of hepatocyte nodules by fibrous bands
Inflammation grading: 1=mild, 2=moderate without giant cells or granulomas 3=moderate with giant cells or granulomas, 4=severe with or without giant cells or granulomas.

Table IIIc: Quantitation of Fibrosis and Inflammation in Liver Sections without Refractile Particles: 14 Biopsy Cases*

Case	Fibrosis	Inflammation	Duration of HD	Remarks
2107/76	0	0	1 mth	
2496/76	0	1	1 mth	
2536/76	0	0	1 mth	
3008/76	0	0	2 mth	
3069/76	0	0	3 mth	centrilobular congestion
3352/76	2	0	2 mth	
3673/76	0	0	2 mth	
3825/76	3	4	3 mth	acute alcoholic hepatitis
4557/77	1	1	2 mth	
107/78	3	3	2 mth	acute alcoholic hepatitis
710/78	1	2	3 mth	metastatic Ca.
356/79	0	0	2 mth	
681/79	1	2	2 mth	
4053/79	0	1	6 mth	

* Liver biopsies from patients serologically positive for HBsAg were not included in this table as HBsAg per se may be associated with hepatic morphologic abnormality

HD = haemodialysis

grading of fibrosis and inflammation as in text and Tables IIIa & IIIb

Table IIId: Quantitation of Fibrosis and Inflammation in Liver Sections without Refractile Particles: 9 Autopsy Cases

Case	Fibrosis	Inflammation	Duration of HD	Remarks
A83/77	3	1	4 mth	
A201/77	0	0	2 mth	centrilobular necrosis
A145/78	2	3	6 mth	alcoholic hepatitis
A266/78	1	1	6 mth	
A15/80	0	0	1 mth	
A36/80	0	0	1 mth	
A192/80	2	0	3 mth	
A231/80	1	1	7 mth	severe fatty change
A275/80	1	0	2 mth	moderate fatty change

HD = haemodialysis

grading of fibrosis and inflammation as described in text and in Tables IIIa and IIIb.

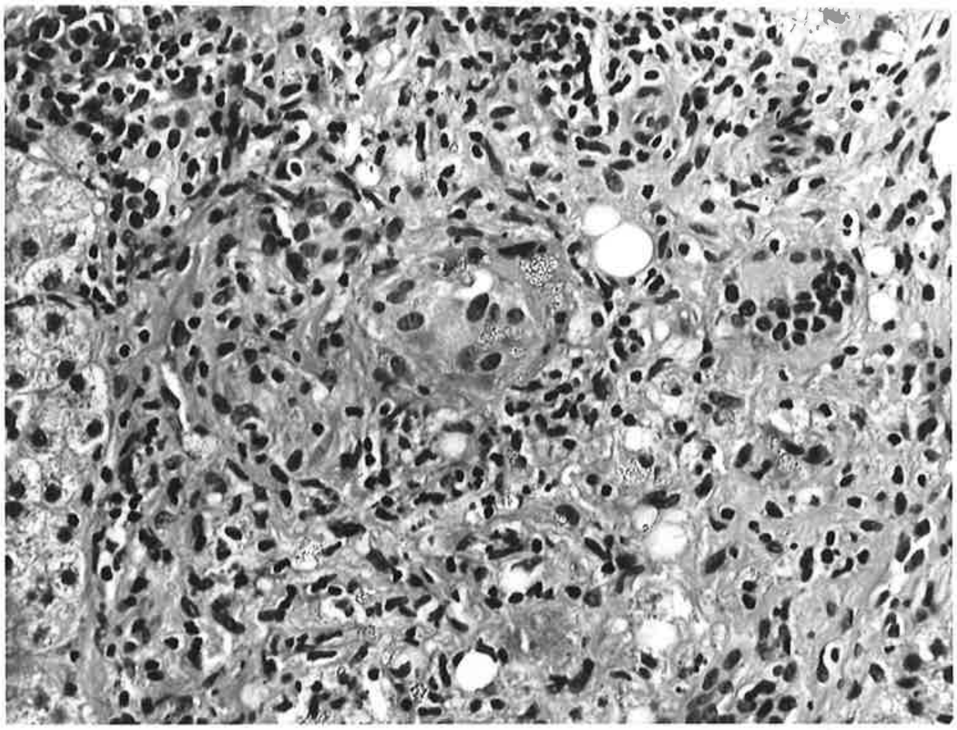


Figure 9: Granulomatous inflammation in portal area. Giant cells with refractile particles, epithelioid histiocytes, lymphocytes and fibroblasts are seen (H & E, x 500).

ground-glass hepatocytes in his biopsy while the other was negative for the antigen (Biopsy Case 17). In the latter, the disruption of the limiting plate was focal and associated with a marked granulomatous inflammation (Figure 10). Scattered macrophages containing clumps of refractile material could also be seen in the hepatic lobules and around central veins (Figure 11).

In no instance was the presence of the refractile particles in the liver associated with normal hepatic morphology. Although a few cases showed no inflammatory response, all liver samples which contained the contaminant had increased amounts of fibrous tissue. The fibrosis was of varying degrees and was essentially located in the portal areas. In two cases (Autopsy Case 20 and 22) fibrosis was so severe as to produce focal areas where nodules of hepatocytes were completely surrounded by fibrous bands (Figure 8d). The encircled nodules, however, mostly retained central veins and appeared to result from bridging of portal tracts to portal tracts rather than portal tracts to central veins. Furthermore, the changes were focal hence not constituting cirrhosis. Autopsy Case 9, however, did have micronodular cirrhosis with fatty change, the aetiology of which was confirmed by a strong clinical history of alcohol abuse.

Splenic Pathology

All the 22 autopsy cases which revealed accumulations of the refractile contaminant within the livers also had the material in the spleen (Table IV). The spleen appeared to accumulate as much of the contaminant as the liver, if not more. The particles were mostly seen around arteries in the white pulp (Figure 12) where they occurred in macrophages and giant cells, as well as freely in the walls of the vessels or in areas of periarteriolar fibrosis. Occasional aggregates of epithelioid cells and giant cells were also seen in these locations (Figure 13) and scattered macrophages in the red pulp also contained the particles.

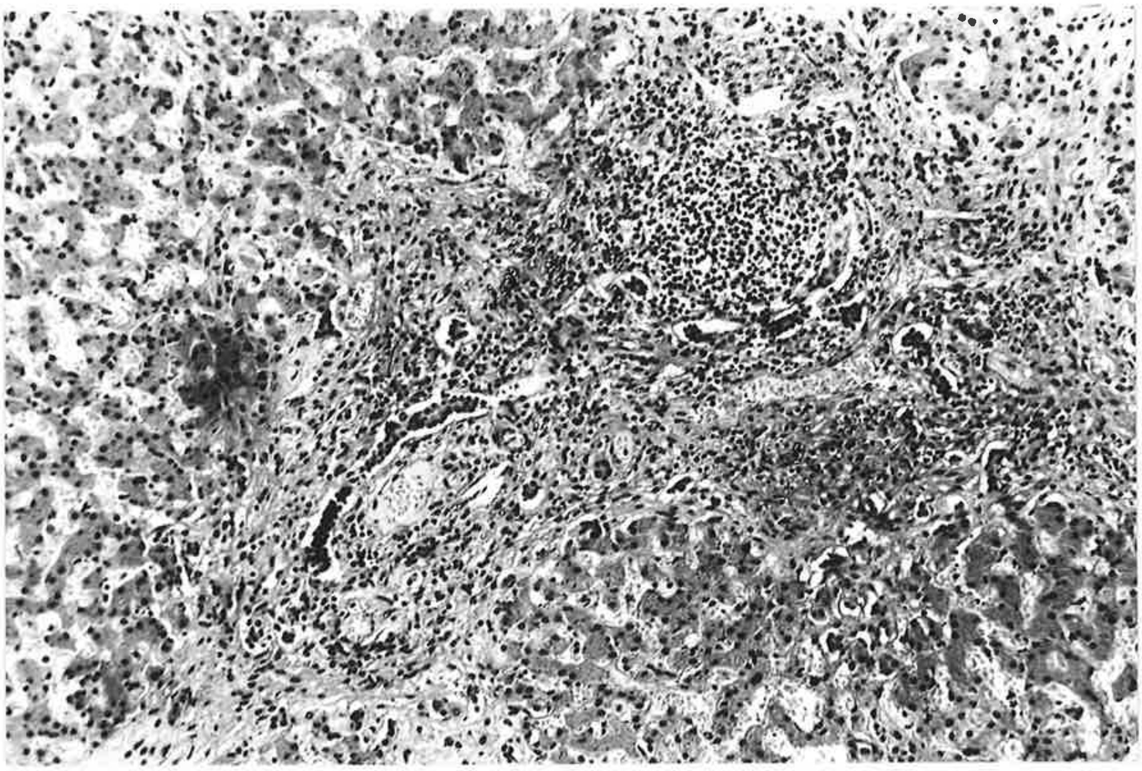


Figure 10: Granulomatous inflammation with focal disruption of limiting plate (Biopsy Case 17, H&E, x 250).

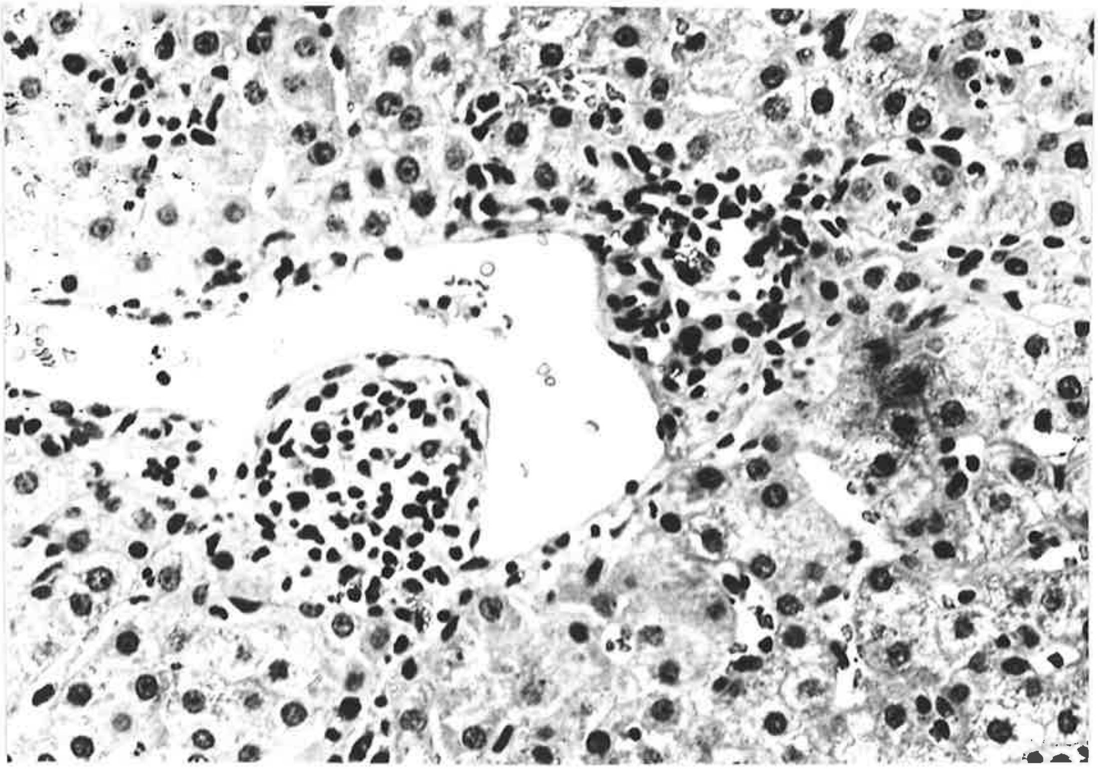


Figure 11: Foci of lymphocytes and macrophages with refractile particles around a central vein (H&E, x 550).

Table IV: DISTRIBUTION OF REFRACTILE PARTICLES IN 22 PATIENTS TREATED WITH REPEATED HAEMODIALYSIS

Case No.	Liver*	Spleen	Bone Marrow	Lung	Lymph Nodes	Remarks
1	1	+	-	-	NA	metas. Ca.
2	1	+	-	-	NA	
3	1	+	-	-	NA	
4	3	+	-	-	NA	
5	1	+	-	-	NA	
6	4	+	-	-	NA	
7	1	+	-	-	NA	
8	1	+	-	+	NA	
9	1	+	-	-	NA	alcoholic cirrhosis
10	2	+	-	-	NA	
11	3	+	+	+	+	
12	3	+	+	+	NA	
13	3	+	+	-	NA	
14	3	+	-	-	NA	
15	3	+	+	+	NA	
16	2	+	-	-	NA	
17	4	+	+	-	NA	
18	3	+	+	+	NA	
19	2	+	-	-	NA	
20	3	+	+	+	+	
21	3	+	+	+	NA	
22	4	+	+	+	NA	limited autopsy

* = refractile particles in liver graded as: 1=barely visible, 2=readily visible, 3=clumped aggregates, 4=confluent clumps

NA=not available

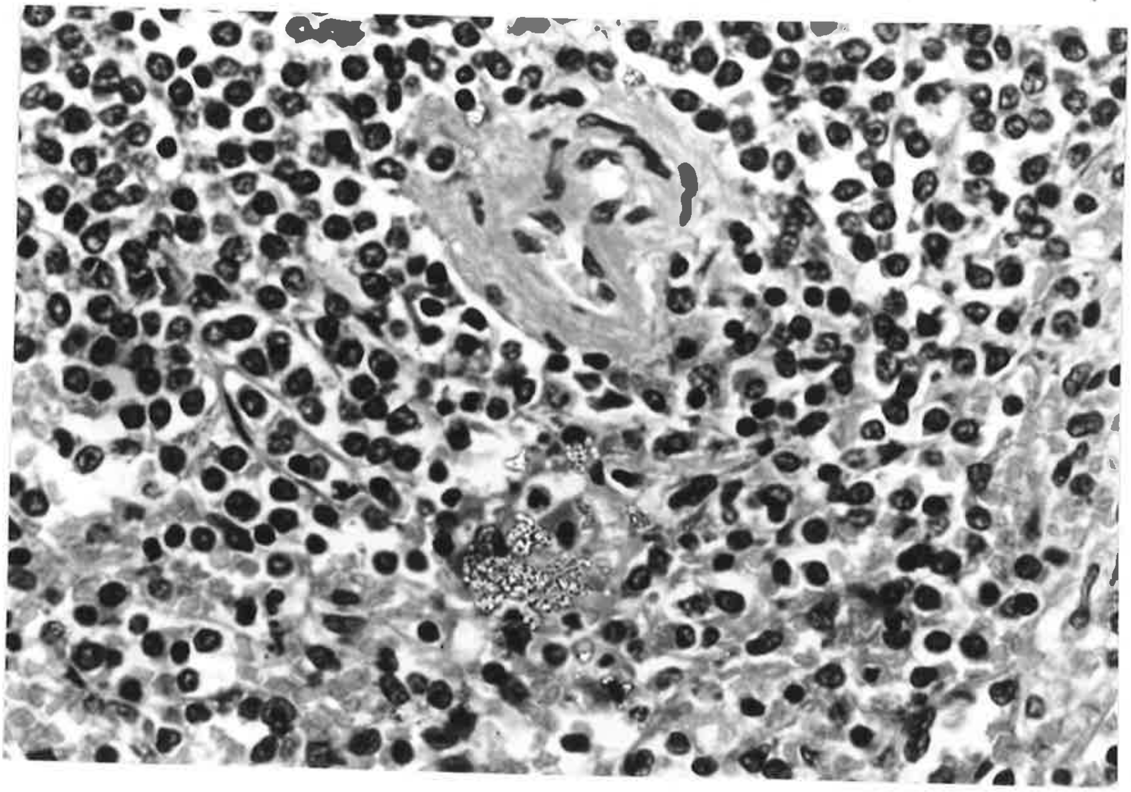


Figure 12: Spleen. Prominent macrophages containing refractile particles are seen in the splenic white pulp. (H&E, x 800).

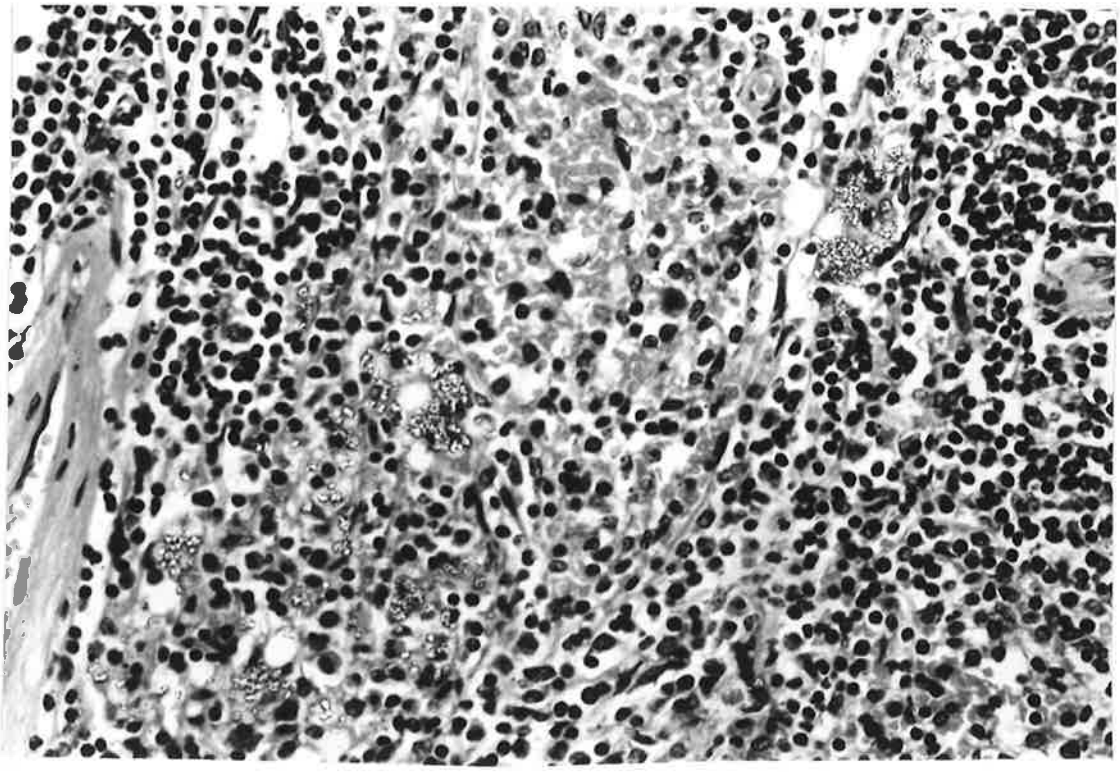


Figure 13: A cluster of epithelioid cells and macrophages with prominent refractile material is evident in the peri-arteriolar area (H&E, x 230).

Lymph Node Pathology

In the only two autopsies where lymph nodes were available for examination, a granulomatous inflammation was found in association with the particles. In both instances the autopsies had been prosected by the author and abdominal as well as mediastinal nodes were found to show sinuses distended with epithelioid histiocytes and scattered multinucleated giant cells both of which contained prominent refractile particles (Figure 14). The latter were also frequently observed in large lipid spaces (Figure 15) and occasional loose clusters of epithelioid cells and giant cells were seen in areas of fibrosis in the medullary and paracortical areas (Figure 16). Macrophages in subcapsular sinuses seldom contained the particles. There appeared to be no significant follicular hyperplasia associated with the presence of the particulate contaminant.

Bone Marrow Pathology

Bone marrow sections were available in 20 out of the 22 autopsies which were positive for the refractile particles. Of the sections examined nine cases showed bone marrow involvement. The material was present in only small quantities which frequently could be detected only by phase contrast microscopy. It was seen in macrophages and an occasional giant cell and also frequently appeared to lie free in fat spaces (Figure 17). Although many marrows showed depressed erythropoiesis or increased granulopoiesis, there did not appear to be significant morphologic alteration in the vicinity of the isotropic deposits.

Pulmonary Pathology

Two sections of lung were available from each of the 22 postmortems and the particles were found in 8 cases. Particles were seen in macrophages and giant cells within capillaries of alveolar septae as well as in alveolar macrophages (Figure 18). Occasionally large flakes

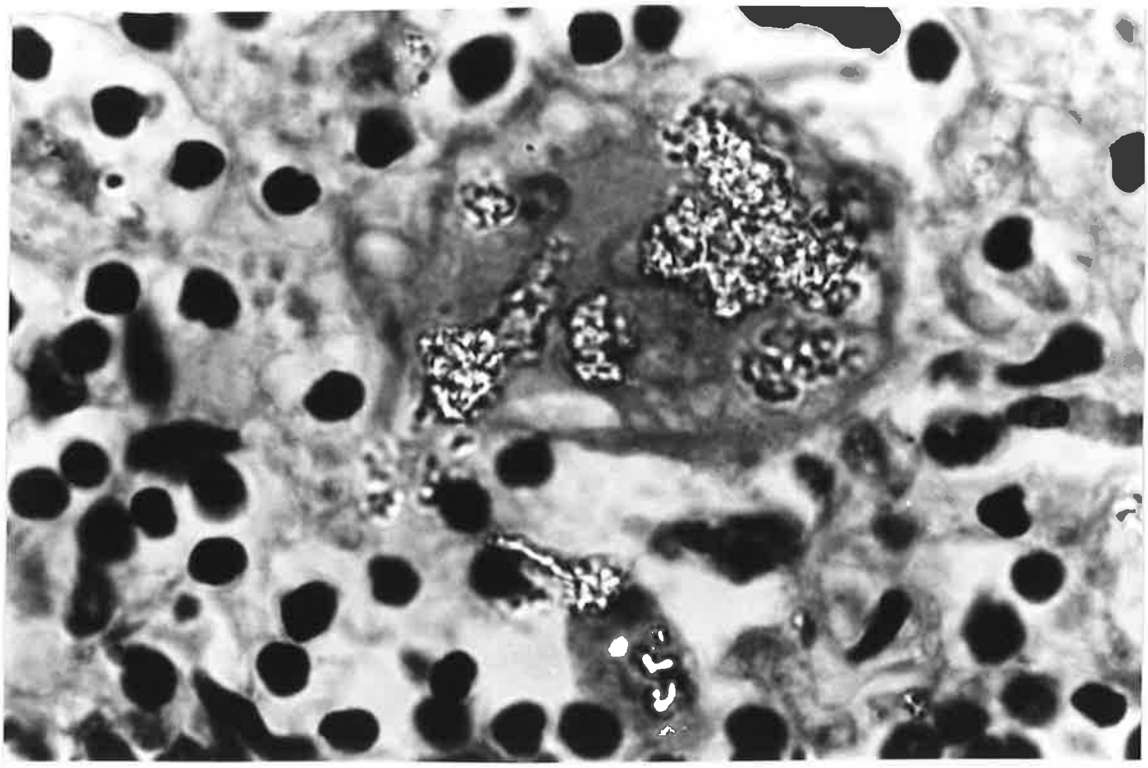


Figure 14: Multinucleated giant cell in a lymph node with striking refractile inclusions (H&E, x 1,500).

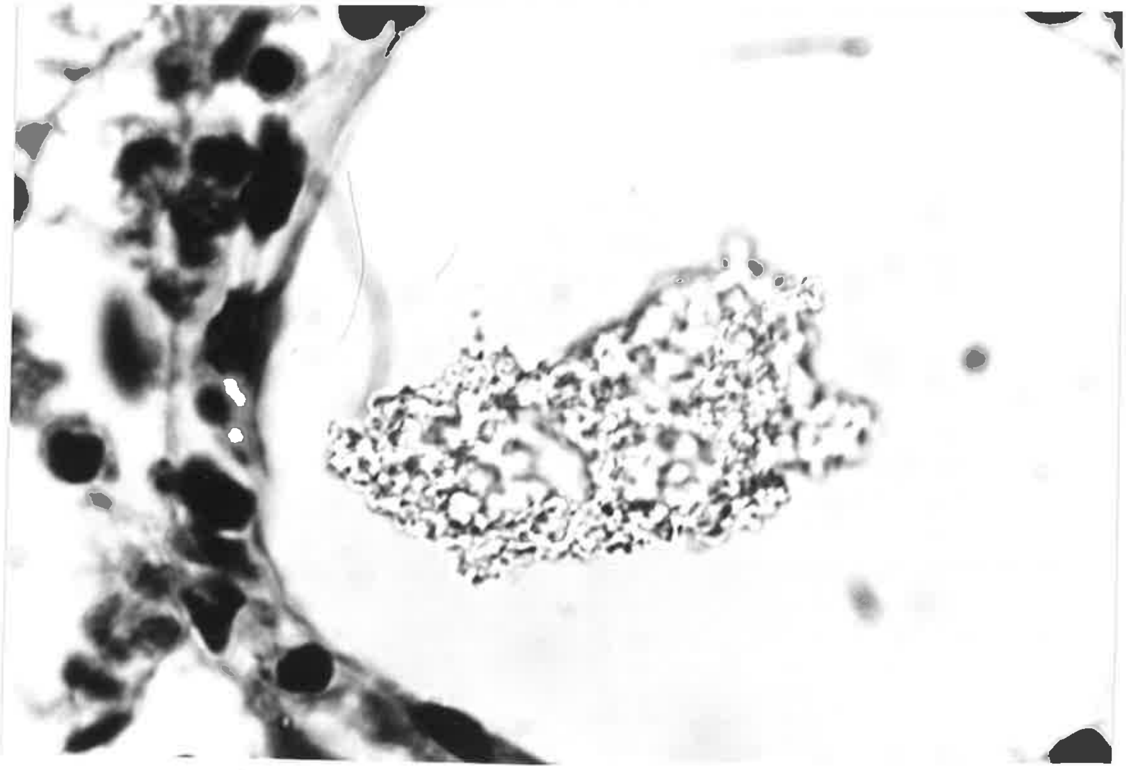


Figure 15: A piece of refractile material lying in an empty fat space. Note the irregular crinkled outlines (H&E, x 1,500).

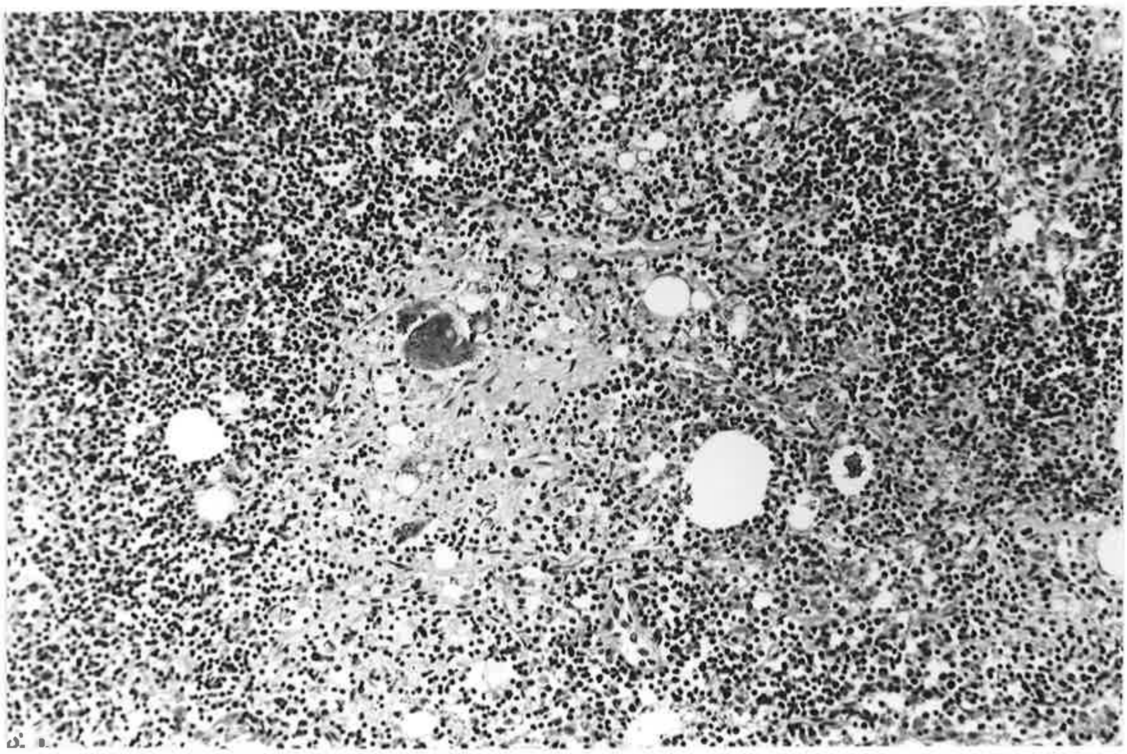


Figure 16: Areas of fibrosis containing epithelioid cells and giant cells with particulate contaminant are seen in the cortical and paracortical areas (H&E, x 250).

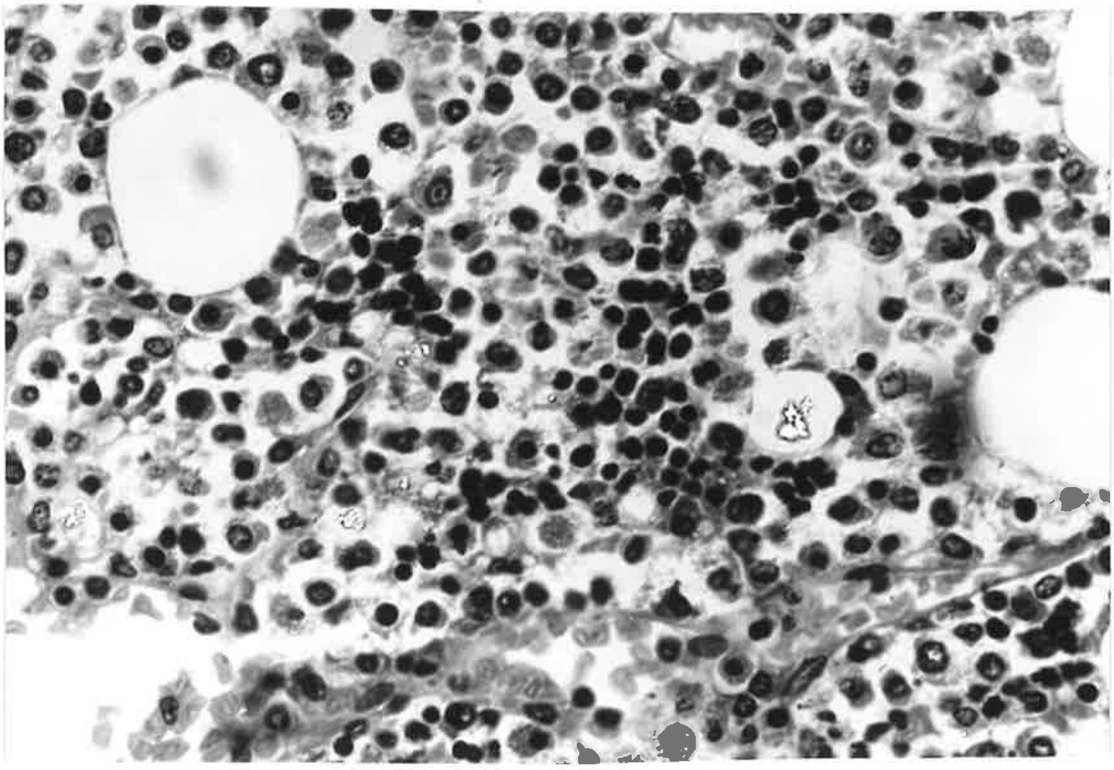


Figure 17: Bone marrow showing refractile particles in macrophages and within fat spaces (H&E, x 500).

were seen in congested alveolar vessels (Figure 19); however, granulomas and fibrosis were not accompanying features.

Other Tissues

It became evident that besides being widely distributed in the mononuclear phagocytic system (reticulo-endothelial system) the colourless, transparent particles were also seen in pulmonary vessels and hepatic sinusoids. Because of this vascular dissemination a careful search for the refractile particles was made in renal glomeruli, brain, adrenals and other vascular organs. Particular attention was also paid to the endocardium of the heart in the event that impaction of the circulating microparticles occurred with the stimulation of endocardial fibrosis. Examination of these and all other autopsy tissues by phase contrast microscopy did not reveal the presence of the contaminants.

IV.b. ELECTRON MICROSCOPIC OBSERVATIONS

Ultrastructural examination was performed on 10 autopsy livers and two spleens. In only two cases were the liver and splenic samples obtained freshly at autopsy and fixed directly in glutaraldehyde. In all other cases wet formalin-fixed tissue was retrieved for processing for electron microscopy. Because of the postmortem delays and the nature of the fixative, preservation of ultrastructural features was generally poor. Despite this it was still relatively easy to recognise the particulate contaminants in the tissue sections.

In the one-micron-thick plastic embedded sections it was noted that by phase contrast microscopy the material lost most of its brightly contrast negative characteristics. Differing brightness is dependent on the difference between the refractive index of the embedding medium and the object of study. The loss of brightness therefore indicated that the refractive index of the microparticles

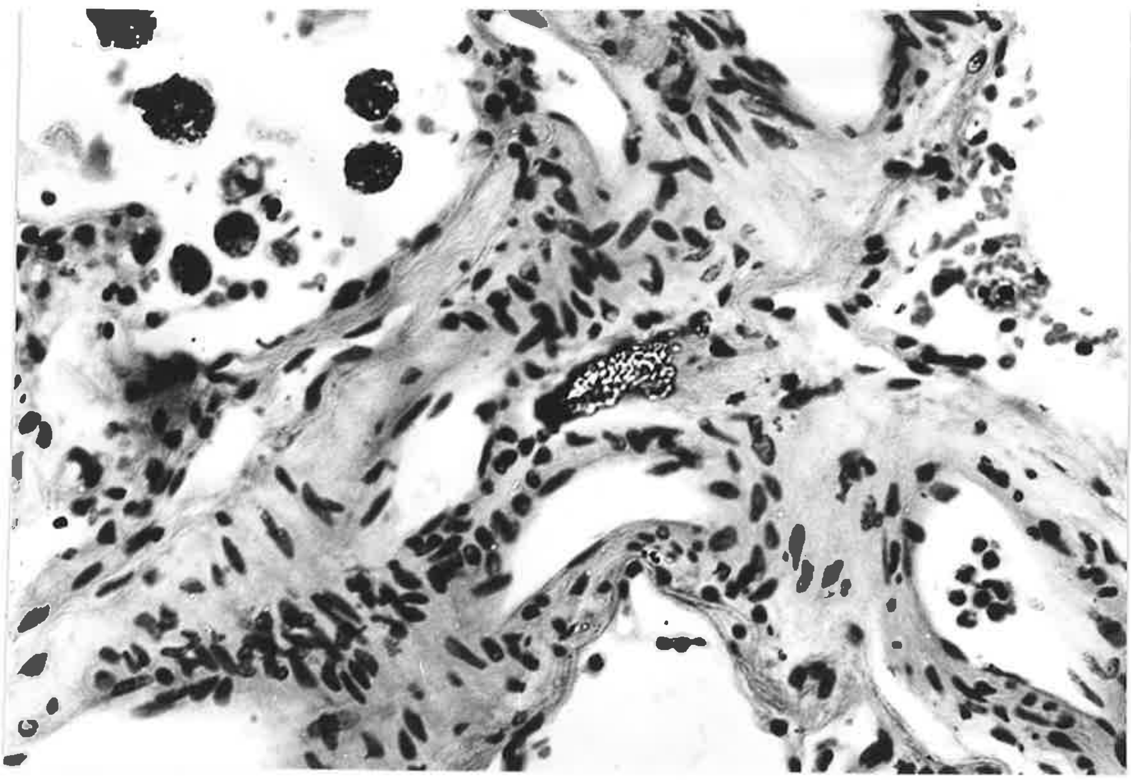


Figure 18: Multinucleated giant cell with granular refractile material seen in a pulmonary vessel (H&E, x 500).

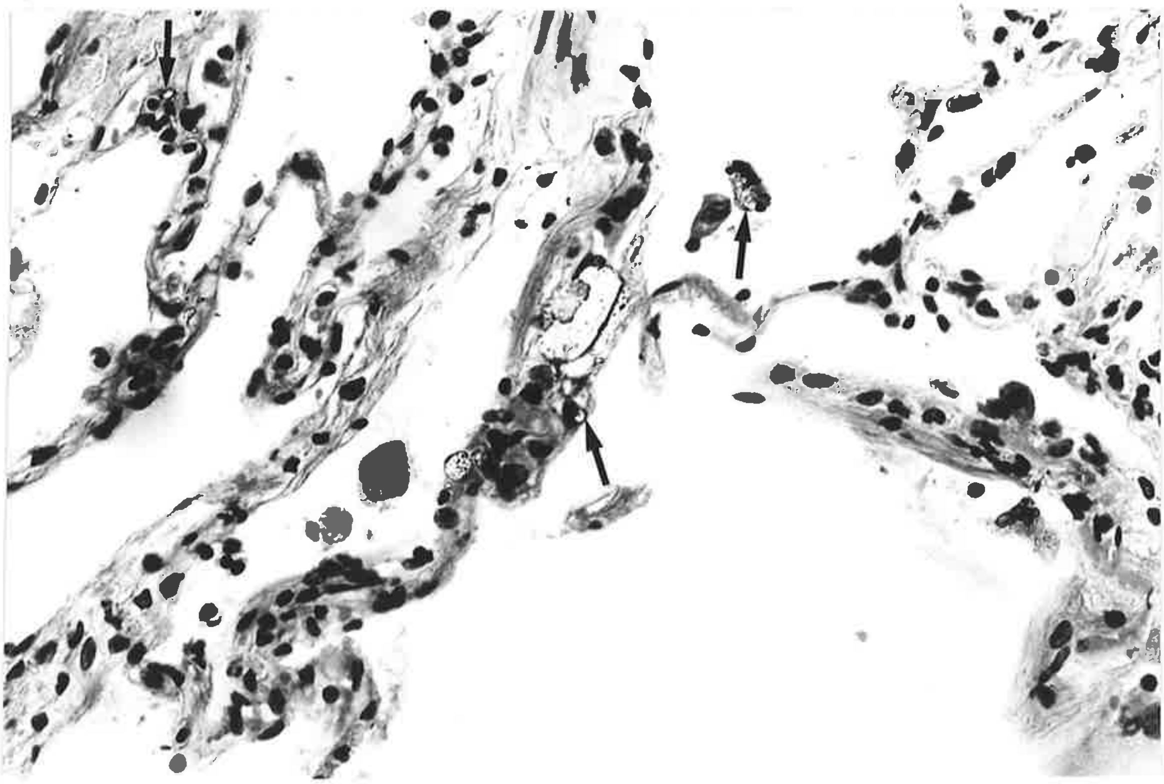


Figure 19: Large piece of refractile material seen in a pulmonary vessel. Smaller particles are also seen in intravascular and alveolar macrophages (arrows) (H&E, x 500).

was very similar to that of the plastic resin.

Ultrastructural Observations in the Liver

A preliminary description of the ultrastructural features of the contaminant has been given in Section II.e. The electron-lucent material was found intracellularly in macrophages and giant cells. It was membrane-bound and appeared to be contained in phagolysosomes not infrequently in association with other phagocytosed material (Figure 20). Although of uniform electron lucency, membranous outlines could be seen within the rounded inclusions (Figure 21). Because of their concentric arrangement these membranes occasionally assumed the appearance of "thumb print" bodies especially when present adjacent to enlarging "burn" holes (Figure 22). The "burn" holes with curling electron-dense margins produced an ultrastructural appearance which came to be recognised as a characteristic feature of the particulate inclusions. Small granular densities were often distributed in the periphery of the particles. There also appeared to be a thin, membrane-like condensation in the periphery of the particles so that when in apposition to lysosomal membranes, two parallel membranes were seen (Figure 23). Larger clumps of granular densities were often found in association with the lucent inclusions in the lysosomes (Figure 24). These deposits were densely packed and difficult to resolve producing an appearance which was consistent with that of compound siderosomes (Ghadially, 1975; Richter, 1978).

The electron-lucent particles were only rarely found lying free in the stroma. They were almost always intracellular in location and in many instances were observed in fragments of degenerating cytoplasm. Despite poor ultrastructural preservation many of the cells containing the contaminants could be recognised as macrophages and the material was not encountered in other types of cells. Plasma cells, lymphocytes, immunoblasts and polymorphs could be found in the vicinity of the inclusion-containing macrophages and collagen and fibroblasts were

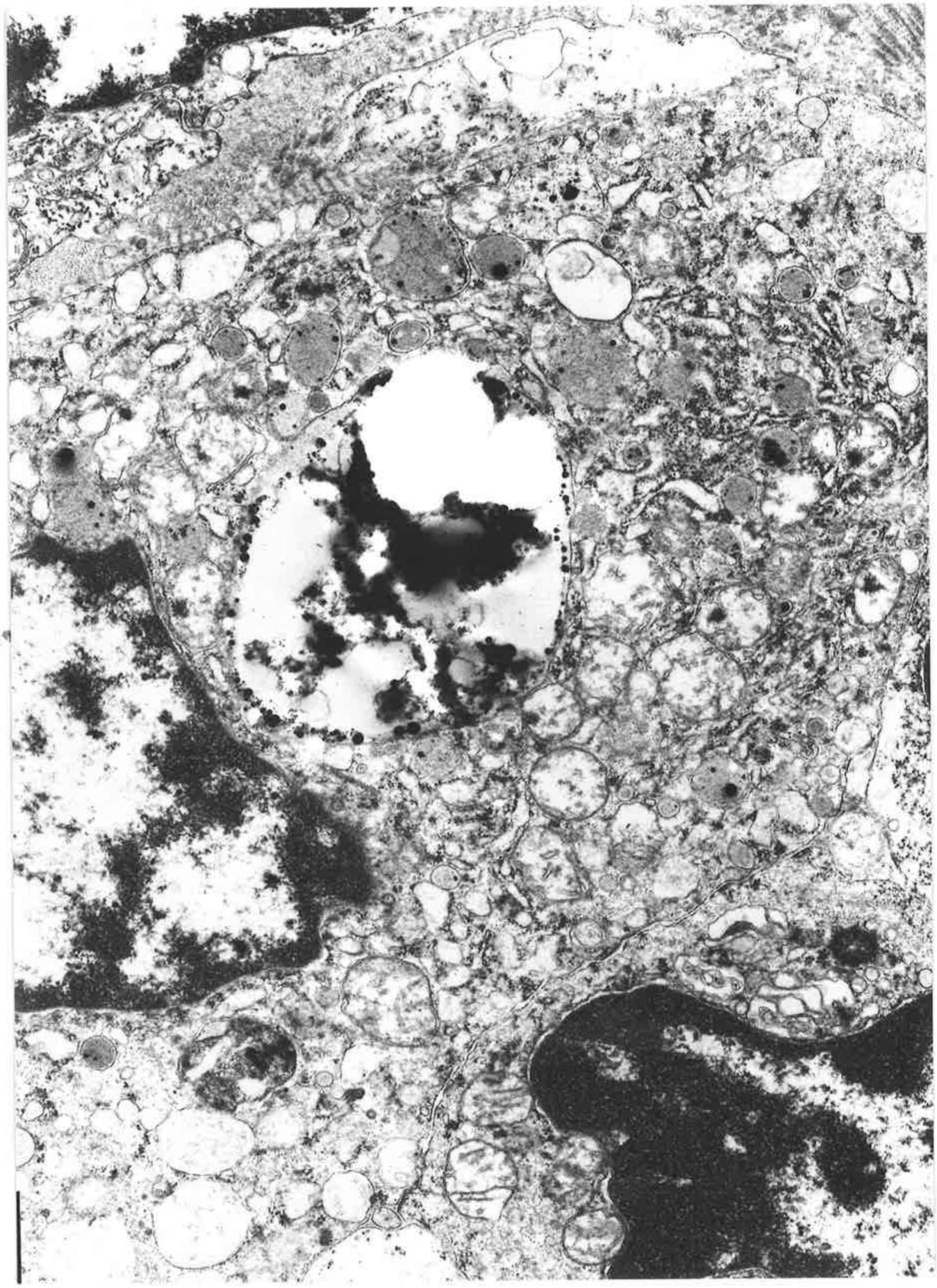


Figure 20: Characteristic lysosomal inclusion in macrophage within portal tract of liver. The material is electron-lucent and shows the characteristic burn hole with curling edges. Granular lysosomal densities are seen in the periphery of the inclusion (uranyl acetate-lead citrate, x 30,000).

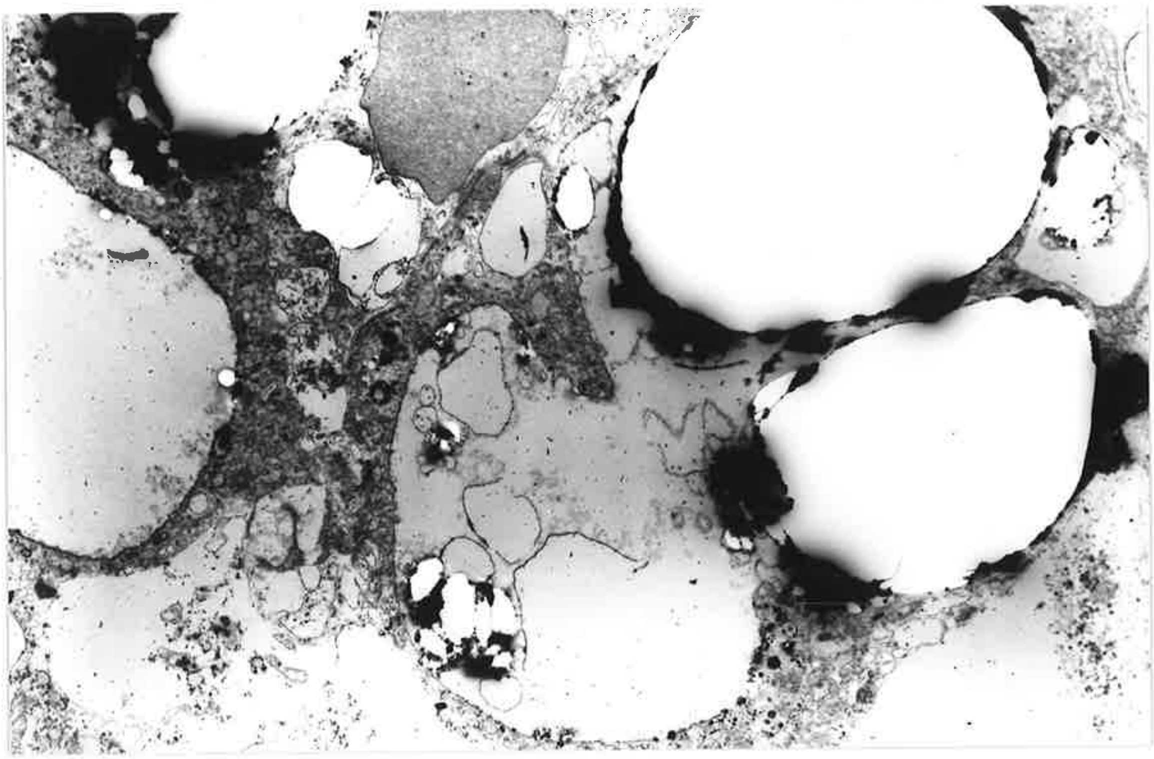


Figure 21: Typical appearance of the foreign material with burn holes and membrane-like structures (uranyl acetate-lead citrate, x 6,000).

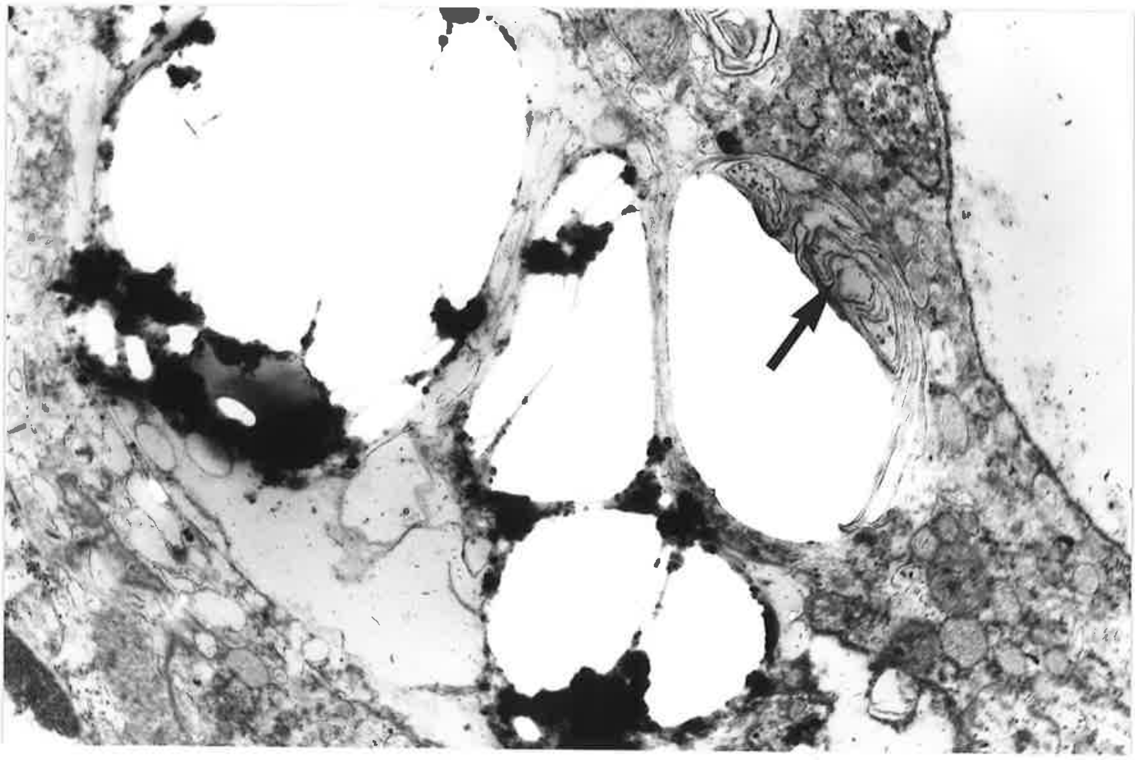


Figure 22: Characteristic electron-lucent inclusion with "thumb print" body adjacent to a burn hole (arrow) (uranyl acetate-lead citrate, x 15,000).

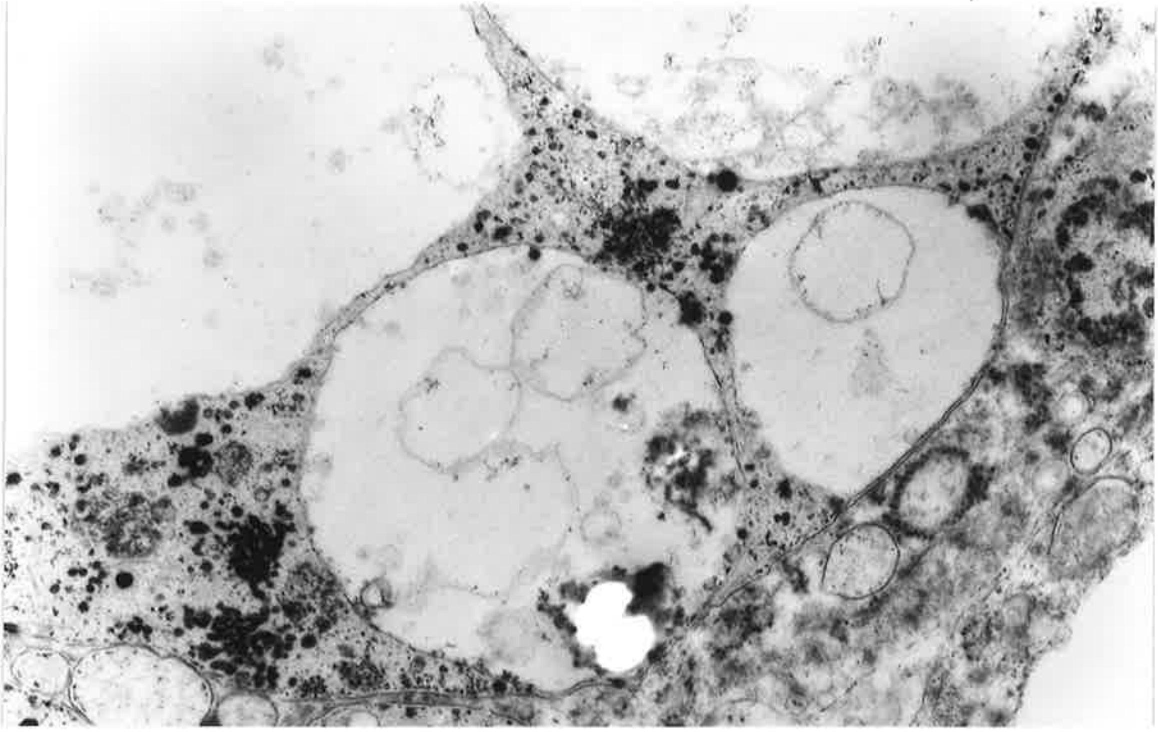


Figure 23: Several electron-lucent inclusions are seen in a hepatic macrophage. Note the membrane-like condensation in the periphery of the inclusions. Compound siderosomes are present in the phagolysosome (uranyl acetate-lead citrate, x 24,000).

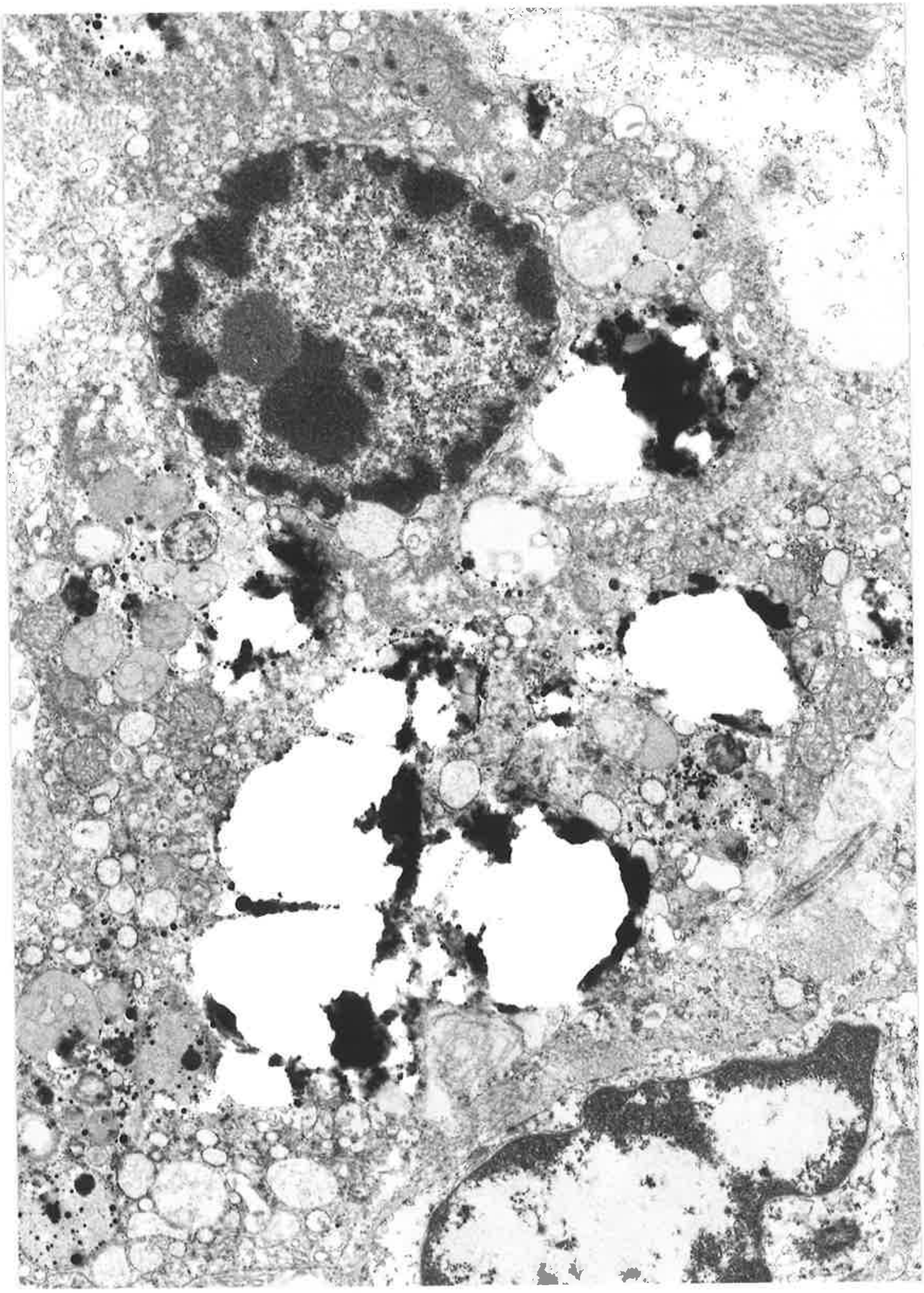


Figure 24: Macrophage in portal area with several characteristic inclusions. Compound siderosomes are also present (uranyl acetate-lead citrate, x 12,000).

also present in the portal tracts. The fibroblasts did not show evidence of myofibroblastic differentiation. The particles were also occasionally sited within fat globules. This was especially prominent in Biopsy Case 12 which showed marked fatty change and round inclusions of lucent material were found in fat-containing macrophages (Figure 25).

An occasional electron-lucent particle was seen in hepatic sinusoids in a few autopsy cases (Figure 26) but this feature was a particularly prominent finding in Autopsy Case 22 in which the observation could be made at light microscopic level (Figure 5). The material was also found in the space of Disse in this patient; however, because of poor preservation it could not be ascertained whether migration of the particles occurred in the presence of an intact sinusoidal endothelium or whether its apparent displacement was an artefact.

Ultrastructural Observations in the Spleen

In the spleen the electron-lucent particles were also found only in phagocytic cells (Figure 27). Membraneous "thumb print" bodies were occasionally seen and the frequency of associated fibrosis and granulomatous inflammation was much less than that observed in the liver.

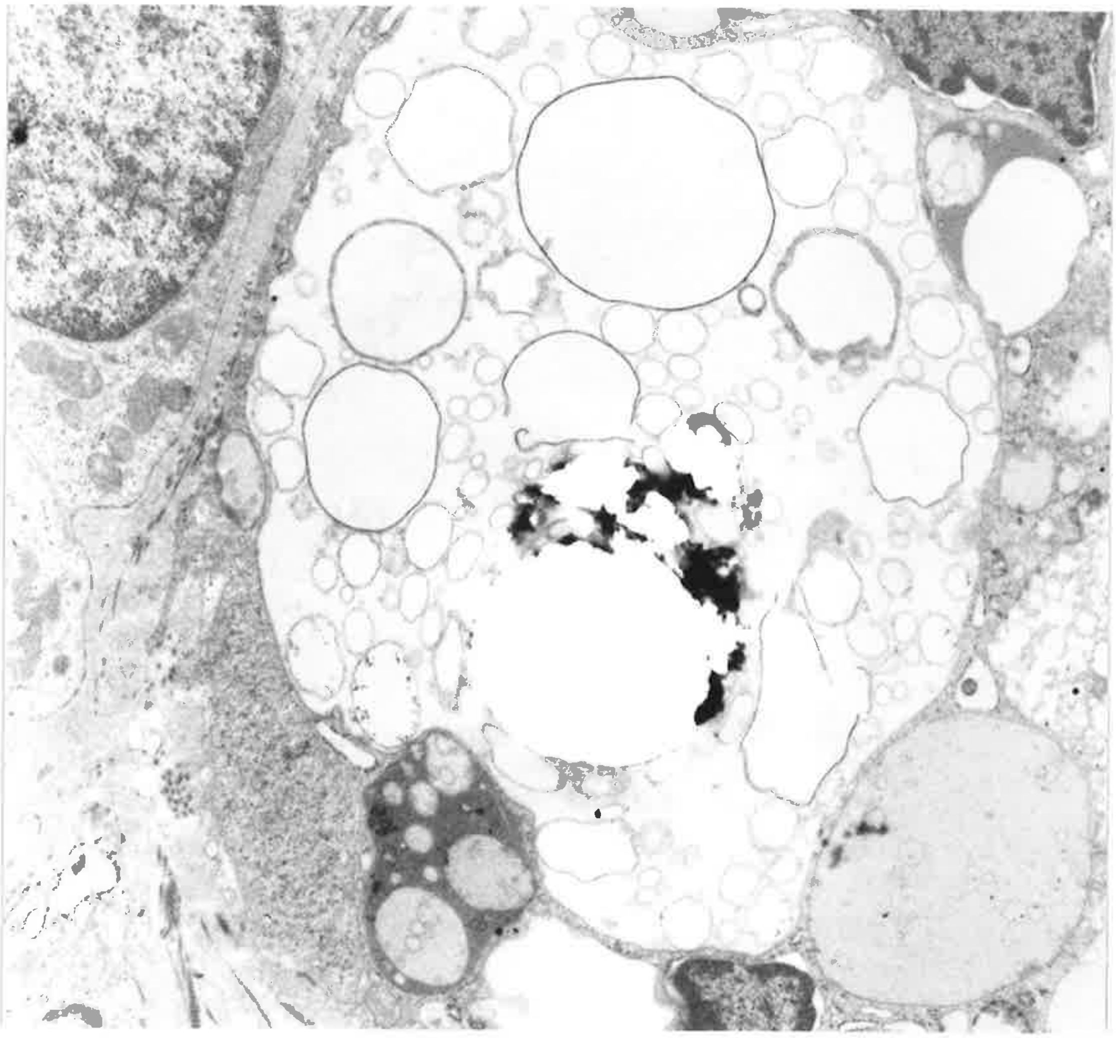


Figure 25: Macrophage containing fat and a piece of the foreign material which shows an early burn hole with a retracting margin (uranyl acetate-lead citrate, x 12,000).

Figure 26: Two small electron-lucent particles are present in the hepatic sinusoid together with several erythrocytes. The fine structural preservation is poor as this is retrieved autopsy tissue (uranyl acetate-lead citrate,

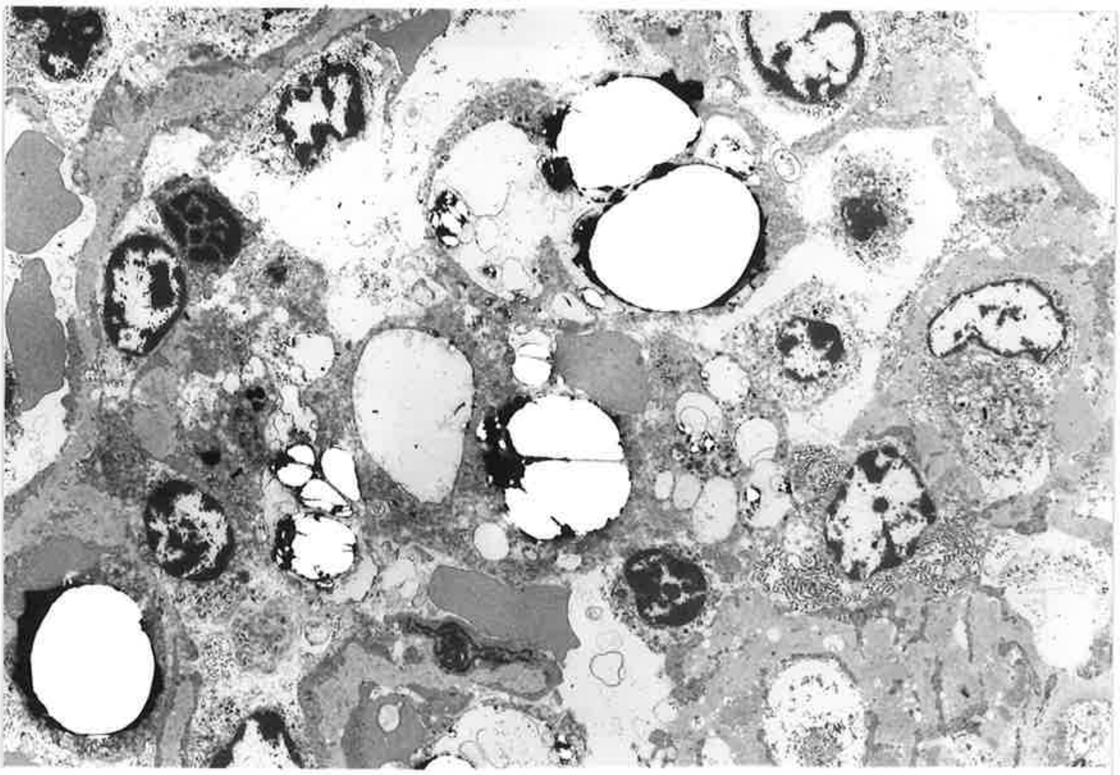


Figure 27: Macrophages in the spleen with the characteristic inclusions (uranyl acetate-lead citrate, x 2,400).

V. PRELIMINARY ANALYSIS OF THE FOREIGN MATERIAL

V.a. LITERATURE REVIEW

Granulomatous Hepatitis
Hepatitis in Renal Patients
Particulate Contamination in Haemodialysis
Methods of Identification of Particulate Material

V.b. CONSULTATION AND PRELIMINARY ANALYSIS

Electron Dispersive X-Ray Analysis
Gas Chromatography-Mass Spectrometry Analysis
Examination of Effluent Blood from Haemodialysis Machines

V. PRELIMINARY ANALYSIS OF THE FOREIGN MATERIAL

Morphologic observations thus far confirmed that the observed refractile material was a particulate contaminant which was found only in patients who had been treated by haemodialysis. The colourless material had a refractive index very similar to that of epoxy resin and its ultrastructural characteristics suggested a further resemblance to plastic. From a pathologic standpoint it seemed that the presence of the material was invariably associated with fibrosis or a chronic inflammation in the liver, which, in some instances,was of a granulomatous nature with prominent giant cells. The material showed evidence of vascular dissemination and was widely dispersed in the mononuclear phagocytic system.

V.a. LITERATURE REVIEW

Granulomatous Hepatitis

Granulomatous inflammation was recognised as a distinct entity in the early nineteenth century and has been of continuing interest since (Long, 1965). Current definitions of granuloma range from an inflammation characterised by the presence of lymphocytes, monocytes and plasma cells to reactions identified by the presence of mononuclear phagocytes including epithelioid and giant cells (Ward, 1975; Adams, 1976). In this study granulomatous inflammation was defined as a loose or compact collection of mature mononuclear phagocytes with specialised mononuclear elements such as epithelioid or multinucleated giant cells.

Granulomatous hepatitis is a pathologic entity associated with many systemic and localised diseases. It is estimated to occur in 3-10% of liver biopsies performed at general hospitals (Guickian & Perry, 1966). The causes are legion and contemporary reviews indicate that tuberculosis and sarcoidosis are by far the most common, accounting for 50-60% of patients in some series (Guickian & Perry, 1966; Simon & Wolff, 1973). In as many as 26% of patients, however, the aetiology of the disease remains obscure (Simon & Wolff, 1973; Mir-Madjlessi et al, 1973). Perusal of the listed causes of granulomatous hepatitis (Klatskin, 1976) indicated that many were not applicable to the present series of cases. Although infective causes such as cytomegalovirus (Clarke et al, 1979) and toxoplasmosis (Weitberg et al, 1979) may occur in the patient with renal failure and impaired immune function, these are not known to be associated with the presence of refractile particles.

Disseminated granulomatosis in the liver and lung is well-recognised in drug addicts (Min et al, 1974) and is attributed to insoluble particulate filler materials that are mixed with the narcotics injected intravenously. The filler material consists of talc (magnesium silicate), corn starch, cotton fibers and other yet unidentified refractile and non-refractile materials (Siegel, 1972). Talc inclusions, however, are distinguished from the material observed in the present group of patients by their birefringent crystalline properties when viewed in polarised light. The ultrastructural appearance of talc is quite dissimilar to the electron-lucent inclusions seen in our cases (Buschmann & Mir, 1979).

Barium as used in radiological procedures bears some superficial resemblance to the refractile material seen in our patients. Many radiocontrast media are primarily excreted by the kidneys and impairment of renal function could result in accumulation of these media in body tissues. The possibility of this occurring in the present group of patients was investigated. A preliminary literature survey revealed that most iodine-containing contrast media for excretion urography may be removed by extra-renal pathways. In nephrectomised rats, intravenous Hypaque (sodium and methyl glycamine diatrizoate) has been shown to be concentrated by the liver and excreted into the intestines (Chamberlain & Sherwood, 1966; Catell et al, 1967). Morphologic examination of the contrast media,however, revealed a colourless liquid with only a weak tendency to crystallisation, making this thesis an unlikely possibility.

Thorotrast (thorium dioxide) was widely used as a contrast agent in some parts of the world before its carcinogenic properties were recognised. This amorphous crystalline material is not birefringent or stainable and accumulates in macrophages in areas of hepatic granulomatous inflammation and fibrosis (Horta, 1967; Kaick et al, 1978). By light microscopy it bears strong resemblance to the refractile particles seen in our cases, but, in contrast, it is electron-dense in character (Smoron & Battifora, 1972). Thorotrast has not been used at our hospital; furthermore, autoradiography tests performed on liver tissues from Autopsy Case 17 and 22 which had large amounts of the refractile material showed no evidence of alpha ionising particle emission.

Hepatitis in Renal Patients

Many potential causes of hepatic dysfunction may exist in the patient on haemodialysis and multiple drug therapy. While detailed studies in this group of patients have not been performed, many reports on renal transplant patients are available (Briggs et al, 1973; Sopko & Anuras, 1978; Ware et al, 1979). Several types of liver disease can occur in renal transplant recipients, the most common being acute and chronic hepatitis. The variety of acute hepatitis includes hepatitis A, hepatitis B, cytomegalovirus hepatitis and herpes simplex hepatitis (Sopko & Anuras, 1978). While a cause could be identified for the majority of episodes of acute hepatic dysfunction, the aetiology of most of the chronic hepatitis remains undetermined. Hepatitis B virus was the most frequently identified aetiologic agent and cytomegalovirus was indicated as a presumptive or potentially responsible factor. Azathioprine has been thought to be responsible for some episodes of acute cholestasis but was exonerated as a cause of chronic disease as was hepatitis A virus (Ware et al, 1979). Iron overload has recently been suggested as a potential cause of hepatic pathology (Gokal et al, 1979) but this could readily be dismissed from being an important factor in this study as only three patients revealed significantly increased hepatic iron stores by Perl's stain (Biopsy Case 13 and 16, Autopsy Case 4). Other medications such as vitamin A and androgens, particularly the oral variety, can produce hepatic dysfunction (Leong & Sage, 1977). These factors, however, did not appear to be operative in this series of patients. Routine screening revealed a raised titre to cytomegalovirus in only four cases and a rising titre to herpes simplex virus, another potential cause of chronic hepatitis (Sopko & Anuras, 1978) was found in two instances. Non-A non-B hepatitis viruses have been implicated as important causes of chronic liver disease in renal patients but there are still no reliable serological tests for these agents and the diagnosis is presently made by exclusion of other known causes of chronic hepatitis (Lancet, 1981).

Particulate Contamination in Haemodialysis

There are a number of ways in which particulate matter is delivered to an organism: 1) by surgical introduction, 2) by direct inhalation of particle-laden air, 3) by intravenous solutions, 4) orally, and 5) by extracorporeal therapy. In the context of our patients, the first two modes of entry are not applicable. We have given consideration to the possible introduction of the refractile contaminant via the oral route and intravenous solutions.

The risk of introducing toxic non-biological substances through extracorporeal blood circuits is real. Early workers with atraumatic cardiac pumps suggested that "spalled plastic particles are undoubtedly responsible for much of the heart (eg subendocardial necrosis and "stone heart" syndrome), brain and other organ damage observed in patients following conventional open heart surgery" (Kletschka et al, 1975). Polyvinyl chloride (PVC) and plasticizers used in the manufacture of dialysis tubings, blood bags and other devices involved in the external circulation of blood are potentially toxic. Plasticizers or phthalates which are components of plastic added to the resin to induce flexibility may be easily eluted and have been detected in the tissues and blood of patients on haemodialysis (Jaeger & Rubin, 1972; Ono et al, 1975; Briggs & Brone, 1976; Chen et al, 1979). In recent years, because of their wide usage, a great deal of attention has been paid to the toxicity of plasticizers (Petrick et al, 1977). Experimental sources suggest that leachable products from PVC tubing can result in cardiotoxicity (DeHaan, 1971; Lawrence, Autian & Misra, 1975) and abnormalities of both liver function and histology have been produced in the subhuman primate (Jacobson, Kevy and Gard, 1979) and rats (Moody & Reddy, 1978). Other more "subtle toxicities" such as tissue culture cell death or enhanced growth and changes in antibody reactivity have also been demonstrated (cited by Jaeger & Rubin, 1970). Considering the long term and massive exposure to synthetic dialysis materials, remarkably few instances of toxicity in the clinical situation have been reported. A viral hepatitis-like syndrome developing in six patients following exposure to new PVC tubings during haemodialysis has been attributed to the high level of plasticizer found in the blood (Neergaard et al, 1971). In a later publication the same authors (Neergaard et al, 1975) described their further investigations of this episode. The toxicity of diethylphthalate was investigated and it was concluded that diethylphthalate could not be proven to have caused the non-viral hepatitis in their patients.

Other reports have indicated that skin eruptions resembling porphyria cutanea may be induced by substances exuded from PVC tubings (Thivolet et al, 1977; Perrot et al, 1977; Poh-Fitzpatrick et al, 1978). Poh-Fitzpatrick et al (1978) observed elevated plasma porphyrins in two patients having such skin lesions. Although the molecular weight of uroporphyrin suggests that it is removable by dialysis to a limited extent, no transfer from blood to dialysate could be detected. The authors indicated that the absence of uroporphyrin in dialysate may be due to protein binding or to repulsive forces at the dialyzer membrane surface due to charge effects. The same authors, however, also observed two other patients with similar skin lesions who had normal levels of serum porphyrin. Thus, the role of PVC tubing in the genesis of these skin lesions is as yet unclear. The evidence presented or suggested by these reports is inconclusive and cannot be considered as establishing the causative role of PVC tubing. A recent report of a patient with necrotising dermatitis has also been linked to toxic products from blood tubings (Bommer, Ritz & Andrassy, 1979).

Lewis et al (1978) demonstrated that levels of serum diethylhexyl phthalate increased with longer durations of dialysis. There was also an individual variable ability to metabolise and store diethylhexyl phthalate and hepatic functional impairment seemed to interfere with its metabolism. Diethylhexyl phthalate is normally excreted by the kidney but in anephrics excretion of the plasticizer is by way of the bile duct,if liver function is normal (Baker et al, 1978).

Plasticizers appeared to be a possible identity of the refractile material observed in our patients. Although reference is made to the occurence of plasticizers as particles (Darby & Ausman, 1973; Needham & Luzzi, 1973; Whitlow, Needham & Luzzi, 1974), these are microspheres of liquid plasticizer which are pseudoparticles and not true solids (Darby & Ausman, 1973). The microspheres can be generated by agitating or shaking a fluid like sodium chloride solution in a flexible PVC container; however, the "particles" return into solution during storage (Whitlow, Needham & Luzzi, 1974).

Van Wagenen and associates (1975) and Kjellstrand (1978) have recovered large particles of plastic released during <u>in vitro</u> testing of new blood tubings. Their reports provided the strongest indication of a likely source of the refractile contaminant seen in this study.

The ultrastructural appearance of PVC has been studied in a case of PVC pneumoconiosis (Arnaud et al, 1978). The PVC inclusions were non-homogeneous and were surrounded by an electron-dense membrane whose outlines were irregular. The material was "either granular or of a fluffy appearance". In vitro studies of PVC particle phagocytosis by alveolar macrophages revealed oval bodies of uneven size with irregular outlines, sometimes surrounded by finely granular lysosomal material. By polarised light the particles were non-birefringent and the material could not be stained.

Methods of Identification of Particulate Material in Tissue Sections

Standard textbooks of histochemical techniques (Lillie, 1965; Dury & Wallington, 1967; Luna, 1968; Pearce, 1972) do not discuss the identification of non-stainable material in tissue sections. A review by Johnson (1972) describes methods of analysis for crystalline particles and Crocker et al (1980) advocate the combined use of electron microscopy with X-ray dispersive spectroscopy.

V.b. CONSULTATION AND PRELIMINARY ANALYSIS

Electron Dispersive X-Ray Analysis

It became apparent from the literature search that the material observed as microemboli in haemodialysis patients had not been previously reported. Electron probe microanalysis appeared to be the most promising method of identification of the contaminant. This facility was not available at the Queen Elizabeth Hospital and Dr T M Mukherjee, Head of Electron Microscopy at the Institute of Medical and Veterinary Science was consulted. He kindly examined wet formalin-fixed liver tissue samples known to contain large quantities of the refractile material by electron dispersive X-ray analysis.

In the first instance he reported "high amounts of silicon in the macrophages" but examination of further two specimens from other patients was not successful (Appendix Ia). Professor F N Ghadially, Head of the Department of Pathology at the University of Saskatchewan was unable to recognise the material from its light microscopic and ultrastructural characteristics. His collegue Dr T A Cunningham felt that the material was probably not endogenous in origin (Appendix Ib). Consultation with a panel of experts made available through the editor of "Ultrastructural Pathology" produced three interesting expert opinions. Professor P Dustin, Head of the Laboratory of Anatomic Pathology and Electron Microscopy at the University of Brussels, suggested that the material might be thorotrast (Appendix Ic). This possibility had previously been considered and excluded as the material did not emit ionizing alpha particles. Professor K Lapis, Director of the Institute of Pathology and Experimental Cancer Research at the Semmelweis Medical University, Budapest, felt the "the disclosure of its (the material) nature is of prime sanitary importance. It is important as well, to make this phenomenon in a wide circle of doctors known" (Appendix Id). Professor Lapis subsequently kindly carried out electron dispersive X-ray analysis on wet hepatic and splenic tissues from three of our cases but was unable to detect any foreign material by this method of analysis (Appendix Ie). Professor Fenton Schaffner of the Mount Sinai Medical Center, New York, suggested that the material was probably "a metal in the form of an oxide, phosphate, sulfate or complex salt engulfed and stored in macrophages" (Leong & Gove, 1982). Presentation of our findings at national meetings (Leong et al, 1980; Disney, Leong & Gove, 1981) stimulated interest but did not help identify the contaminant.

Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-mass spectrometry is an established and sensitive method of detecting plasticizers (Hillman, Goodwin & Sherman, 1975). Analysis conducted by Dr G Phillipou of the Endocrinology Laboratories, Department of Obstetrics and Gynaecology, the Queen Elizabeth Hospital yielded no significant difference in levels of plasticizers in three liver samples with the refractile material over that of control liver tissues.

Examination of Effluent Blood from Haemodialysis Machines

Microscopic and ultrastructural examination of the ultrafiltrate of effluent blood downstream of the dialyser as well as dialysate samples did not reveal the presence of any refractile material.

VI. ANIMAL EXPERIMENTS

VI.a. INTRAVENOUS INJECTION OF PVC AND SILICONE

VI.b. SUBCUTANEOUS INTRODUCTION OF SILICONE PARTICLES

VI. ANIMAL EXPERIMENTS

At this stage of the investigation it seemed that the most likely identity of the refractile material was PVC or some other plastic contaminant derived from the blood lines or blood bag. The dialysis filter material, cuprophane, was a possible source of particulate contamination but it was eliminated as a suspect when it was found to have birefringent properties. Examination of blood tubings in dialysis machines revealed that PVC was the most common material in direct contact with the blood. Direct questioning and inquiry revealed that the dialysis unit in the hospital had up to November 1979 used silicone (silastic) as the roller pump tubing segment; however, "decay changes" occurring in the silicone tubing after use necessitated its replacement with PVC.

VI.a. INTRAVENOUS INJECTION OF PVC AND SILICONE

Animal experiments were designed to test if PVC or silicone introduced intravenously would produce granulomatous hepatitis. Tissue examination would also allow study of the morphological and optical properties of these plastics. As a screening procedure filings of PVC from the blood pump tubings were embedded in autopsy tissue and prepared for examination in an electron microscope. The ultrastructural appearance of PVC particles was not dissimilar to that of the electron-lucent contaminants (Figure 28).

An initial group of four adult Wistar rats was chosen for intravenous injection of filed particles of PVC in saline. Attempts at introducing the particles into the tail veins of the animals were complicated by thrombosis of the vessels in all instances, as it was not possible to render the particles sufficiently small. A similar problem was encountered with the use of silicone particles scraped from the tubing.

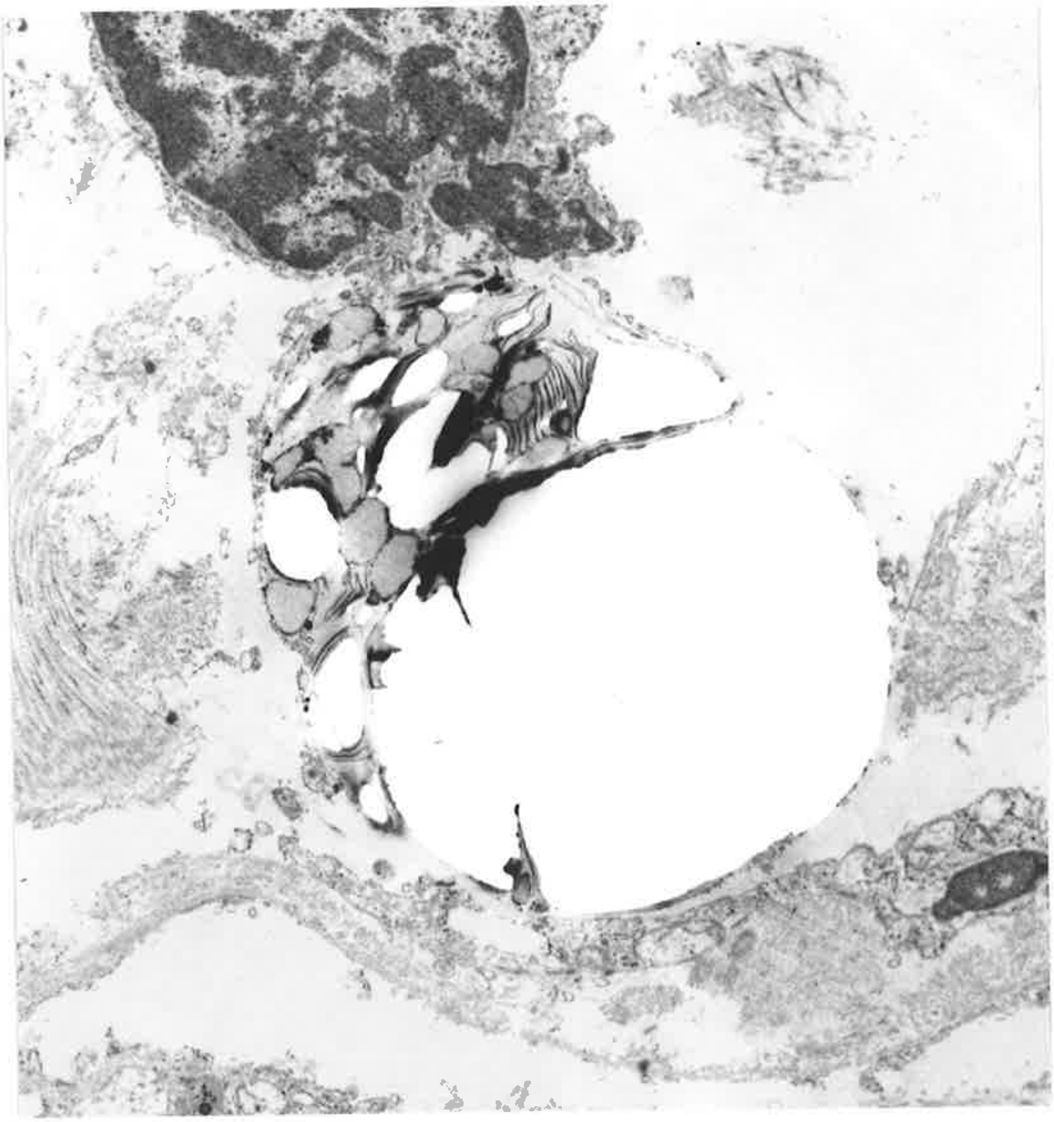


Figure 28: Electron micrograph of PVC filings artificially embedded into a piece of fibrous tissue. Although electron-lucent, PVC is slightly denser that the material seen in the haemodialysed patients. The tendency to burn holes is a common feature (uranyl acetate-lead citrate, x 7,500).

VI.b. SUBCUTANEOUS INTRODUCTION OF SILICONE PARTICLES

A second batch of four adult Wistar rats was selected and the inner aspects of all limbs were shaven and swabbed with alcohol. The external surface of a silicone roller pump tubing was swabbed with alcohol and fragments of plastic were scraped off with a sterile scalpel. These particles were then introduced into four skin incisions made in the cleaned inner aspects of the right limbs of the rats. In two rats the particles were deposited in the subcutaneous layers while in the others the silicone was placed intramuscularly. Similar incisions were made in the opposite limbs but no silicone particles were introduced. All wounds were closed with silk. After 7 days, palpable nodules developed in the areas of silicone deposition. Biopsies were taken from these areas as well as the opposite control sites and prepared for light microscopic and ultrastructural examination. A granulomatous inflammation was found in the areas of subcutaneous deposition of silicone. Large empty spaces, pieces of refractile material, macrophages and giant cells containing granular refractile inclusions were seen (Figure 29). Electron microscopy revealed large electron-lucent inclusions within macrophage lysosomes (Figure 30). In addition, there were also areas of electron density and "membrane-like" structures were noted in the inclusions. Characteristic "burn" holes were also displayed by the inclusions of silicone. The intra-muscular deposits of silicone only evoked a minor chronic inflammatory response and no granulomas were found. The sham-operated sites contained only granulation tissue.

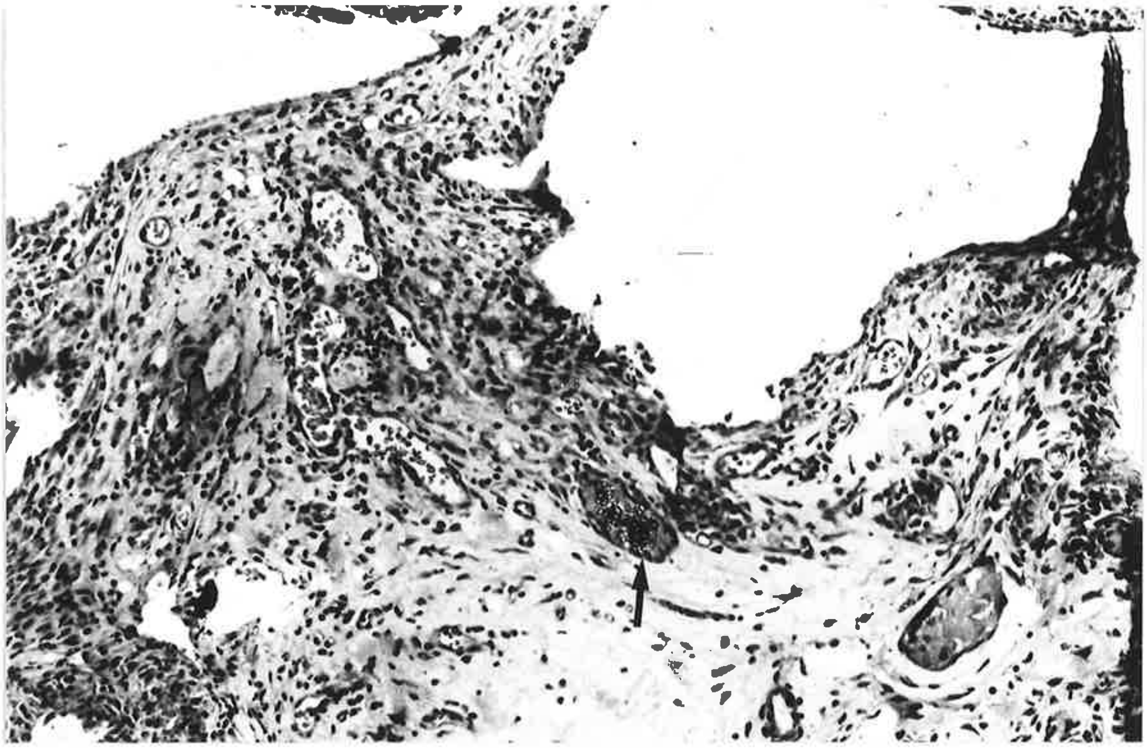


Figure 29: Subcutaneous silicone granuloma in the rat. A granulomatous inflammation is seen around empty spaces which represent places where silicone filings have fallen out of the section. Several giant cells are present, some with refractile inclusions (arrow) (H&E, x 250).

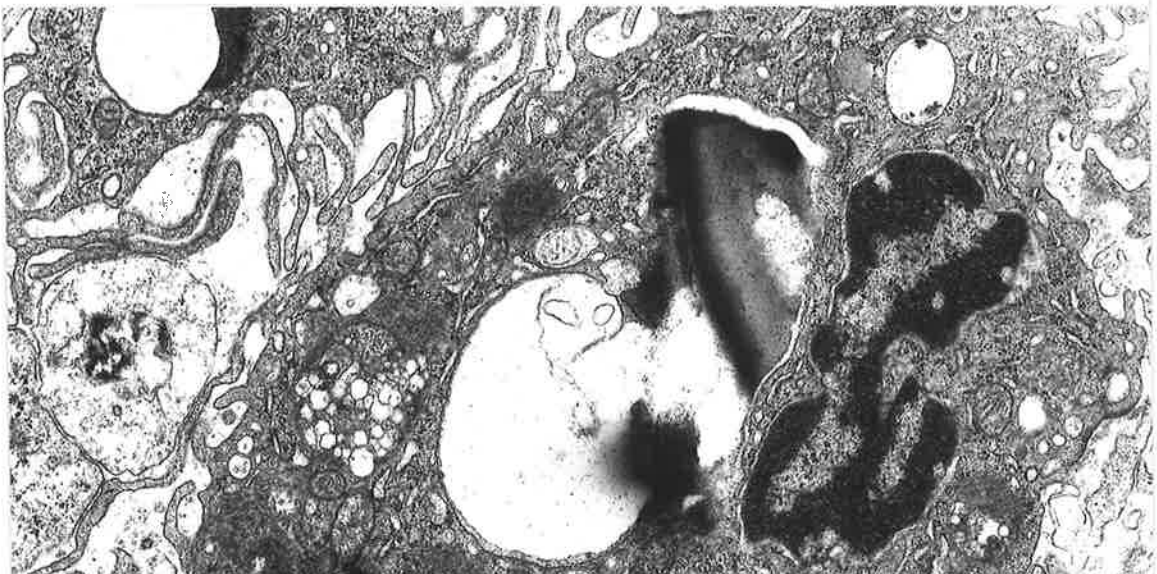


Figure 30: Electron micrograph of rat macrophage containing an electron-lucent inclusion (uranyl acetate-lead citrate, x 8,000).

VII. DEFINITIVE IDENTIFICATION OF THE FOREIGN MATERIAL

VII.a. MORPHOLOGIC EXAMINATION OF SILICONE MASTITIS

VII.b. ELECTRON DISPERSIVE X-RAY ANALYSIS OF THE FOREIGN MATERIAL

VII.c. ATOMIC ABSORPTION SPECTROMETRY ANALYSIS OF THE FOREIGN MATERIAL

VII. DEFINITIVE IDENTIFICATION OF THE FOREIGN MATERIAL

There is currently a great deal of interest in the decay and fragmentation of prostheses manufactured out of silicone. Three reports have described the occurrence of fragmentation of silicone elastomer joint prostheses with production of "dendritic synovitis" and lymphadenopathy (Aptekar et al, 1974; Christie et al, 1977; Kircher, 1980). Both synovium and draining lymph nodes revealed a granulomatous inflammation with multinucleated giant cells which contained a refractile, non-birefringent material very similar to that seen in our patients. In these reports the nature of the foreign material was identified only by its morphological similarity to filings from a new silicone elastomer prosthesis. It was stated that the manufacturers had declared that "at present there is no simple technique for definitely identifying silicone in a lymph node" (Christie et al, 1977). The appearances of these fragments of prosthesis further supported the contention that the microemboli seen in our patients was silicone.

VII.a. MORPHOLOGIC EXAMINATION OF SILICONE MASTITIS

Silicone is widely employed in the field of plastic surgery, especially for the augmentation of breasts. As it happened, biopsies from a case of silicone mastitis were accessioned during the period of this investigation. The material was from a young woman who had had bilateral injections of liquid silicone into her breasts. Fresh biopsy material was fixed in glutaraldehyde and prepared for electron microscopic examination. Tissue was also prepared in the usual manner for light microscopy.

The macroscopic specimen was greasy and contained large blobs of a grey putty-like substance. At the light microscopic level, large empty spaces were found in the tissue sections, representing extra-cellular deposits of the material which had a tendency to fall out of the sections. A prominent chronic inflammatory response was seen in dense fibrous tissue which contained large vacuoles of amorphous, unstainable material and collections of foamy macrophages and giant cells were also present (Figure 31). The substance was non-birefringent and only smaller intracellular deposits were refractile. In the electron microscope, the rounded intracytoplasmic inclusions were electron-lucent and contained membrane-like structures. They were seen in lysosomes of macrophages and were associated with granular lysosomal deposits (Figure 32).

VII.b. ELECTRON DISPERSIVE X-RAY ANALYSIS OF THE FOREIGN MATERIAL

Initial electron dispersive X-ray analysis (EDXA) had been performed by a local and an overseas expert. Although the examination had been made on liver and spleen samples confirmed by light microscopy and ultrastructural studies to contain the particulate contaminant, they were reported to show no foreign material. Despite this initial setback, it was decided to repeat the analysis as EDXA appeared to be the method most likely to reveal the identity of the refractile material.

EDXA is a method of microanalysis which has only in recent years been applied to diagnostic pathology (Ghadially, 1979; Abraham, 1980). The technique consists of focussing a beam of fast electrons onto an area of specimen surface and examining one or more of the signals that emanate due to the interaction. X-ray and electron energy loss signals are typically used for elemental analysis. Other signals that can be used for chemical determination are Auger electrons,

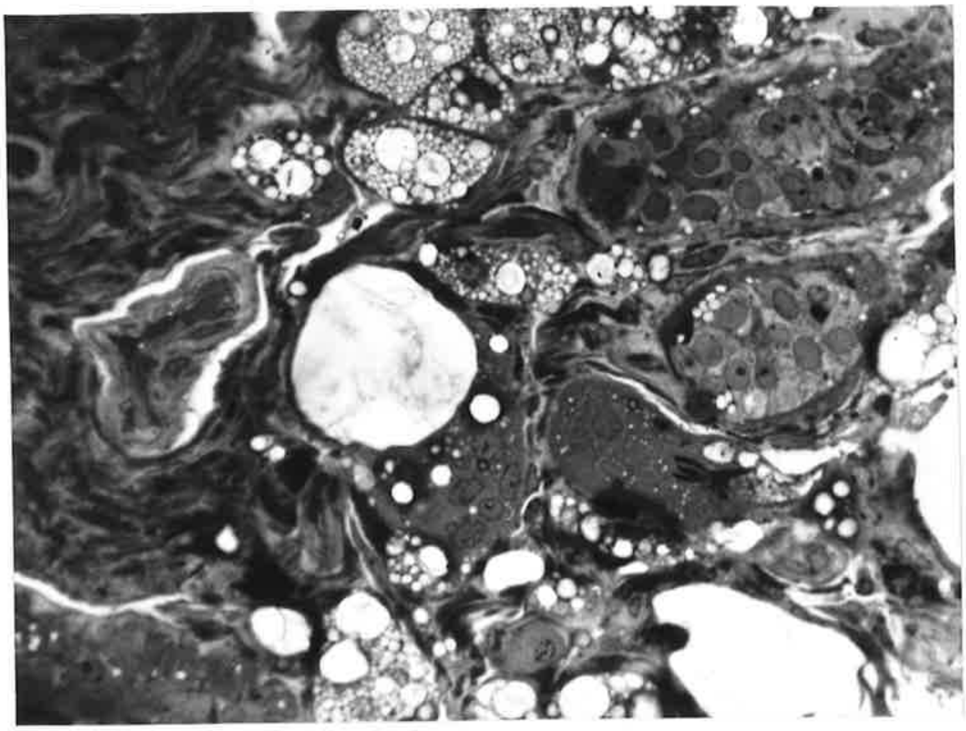


Figure 31: One micron-thick section of silicone mastitis. Macrophages with foamy cytoplasm are seen in the fibrous tissue. A giant cell in the center of the picture contains a large vacuole (toluidine blue, Epon embedded, x 500).

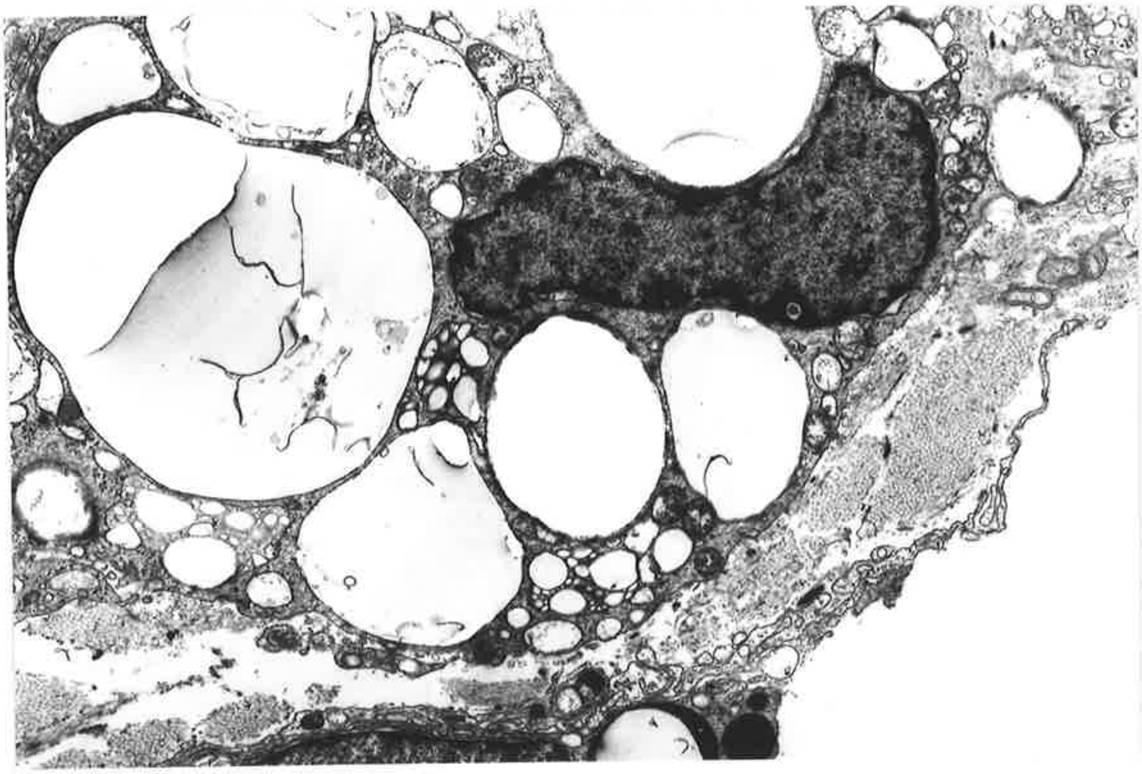


Figure 32: Electron micrograph of a macrophage containing electron-lucent inclusions of silicone from a case of silicone mastitis (uranyl acetate-lead citrate, x 4,000).

fluorescence in the optical region (cathodoluminescence) and electron diffraction and channeling. This microanalytical technique also has the important advantage of working on fresh tissue as well as on material which has been stored for long periods in fixative. It is reported to work on mummified tissue of a thousand years (Abraham, 1980).

EDXA was performed with the assistance of Mr P Schultz of the Geological Services Division, Australian Mineral Development Laboratories. Samples from livers previously subjected to EDXA were studied (Autopsy Case 11 and 22). In addition, a biopsy containing subcutaneous silicone filings from a rat was examined. The tissues were embedded in Epon and coated with carbon to render them electrically conductive. They were examined in a scanning electron microscope equipped with an energy dispersive detector. When first examined, the rat tissue showed high concentrations of silicon and the human livers contained some concentration of the element in areas which were too small to be resolved with the electron probe (Appendix IIa). The analysis was repeated using the technique of back-scattered electron imaging to locate the areas of silicon deposition. The interaction between an incident electron beam and any specimen results in a variety of signals distinct from the scattered electrons, these include Auger electrons, X-rays and back-scattered electrons. Back-scattered electrons emerge in part from the cell surface, but mostly from within the cells. The latter property allows intracellular elements to be detected. When collected by the appropriate detector, these electrons can be used to produce images in the scanning electron microscope - a technique known as back-scattered electron imaging. These images reflect the topographical distribution and concentration of a large number of elements (Fiori, 1981; Soligo & de Harven, 1981). With the aid of this technique it became possible to study the small localised deposits of the foreign material (Figure 33). Spectrometer scans of these areas revealed distinct silicon peaks in both human

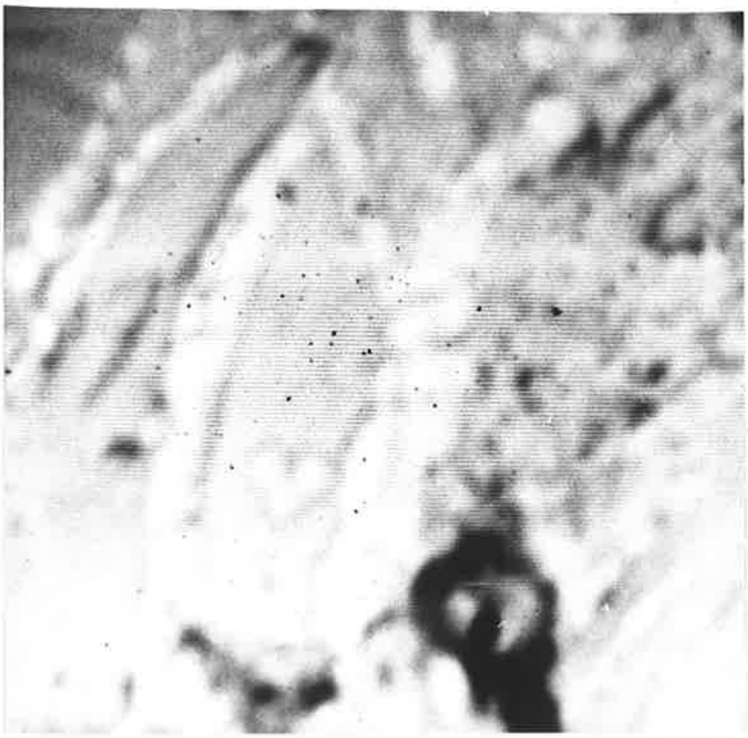


Figure 33a: Scanning electron micrograph of subcutaneous silicone deposits in the rat using the back-scattered electron mode (x 5,000).

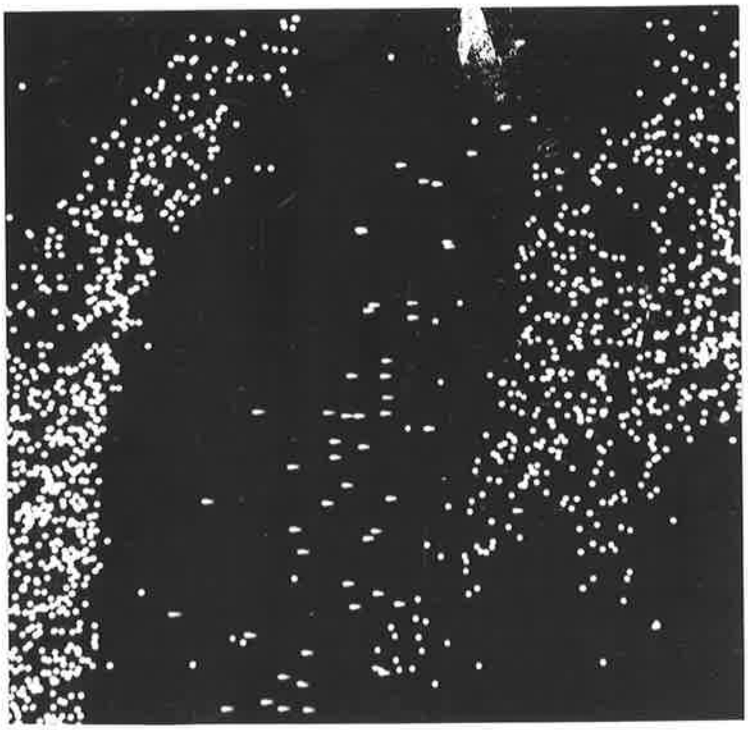


Figure 33b: X-ray distribution map for the silicon present in the same area in Figure 33a. The clusters of white dots represent the location of the silicon (x 5,000).

liver samples and the energy spectra were identical in shape and distribution with that of the silicone filings in the rat tissue (Figure 34) (Appendix IIb).

EDXA identifies the element silicon rather than being specific for the compound silicone (polydimethylsiloxane, a long chain polymer with silicon as its backbone). This analytical technique does, however, combine the morphological demonstration of the material with the elemental analysis which showed the presence of only silicon. This excluded silicates which have other cations eg Na, Mg, Al, K etc. Pure silicon does not naturally occur in the free state and SiO_2 (silica) does not have the optical characteristics of the refractile material. The spectra,furthermore, were identical with those of the pure silicone filings in rat tissue.

It is instructive to speculate why the initial microprobe examinations failed. Neither of the two previous examinations employed back-scattered electron imaging and areas of analysis were based on transmission electron microscopy images. As the foreign material was mostly intracellular and only in small areas, it is conceivable that they could have been missed. Our success in identifying the nature of the contaminant lends support to Abraham's (1980) claim that back-scattered electron imaging with compositional contrast is essential in making efficient diagnostic use of microanalysis in the scanning electron microscope.

Following reports of our preliminary findings (Leong et al, 1981a; 1981b), Professor J L Abraham of the University of California offered to examine our material. Using EDXA with back-scattered electron imaging he confirmed our findings that the particles of refractile material contained high concentrations of silicon (Appendix IIc) (Figure 35).

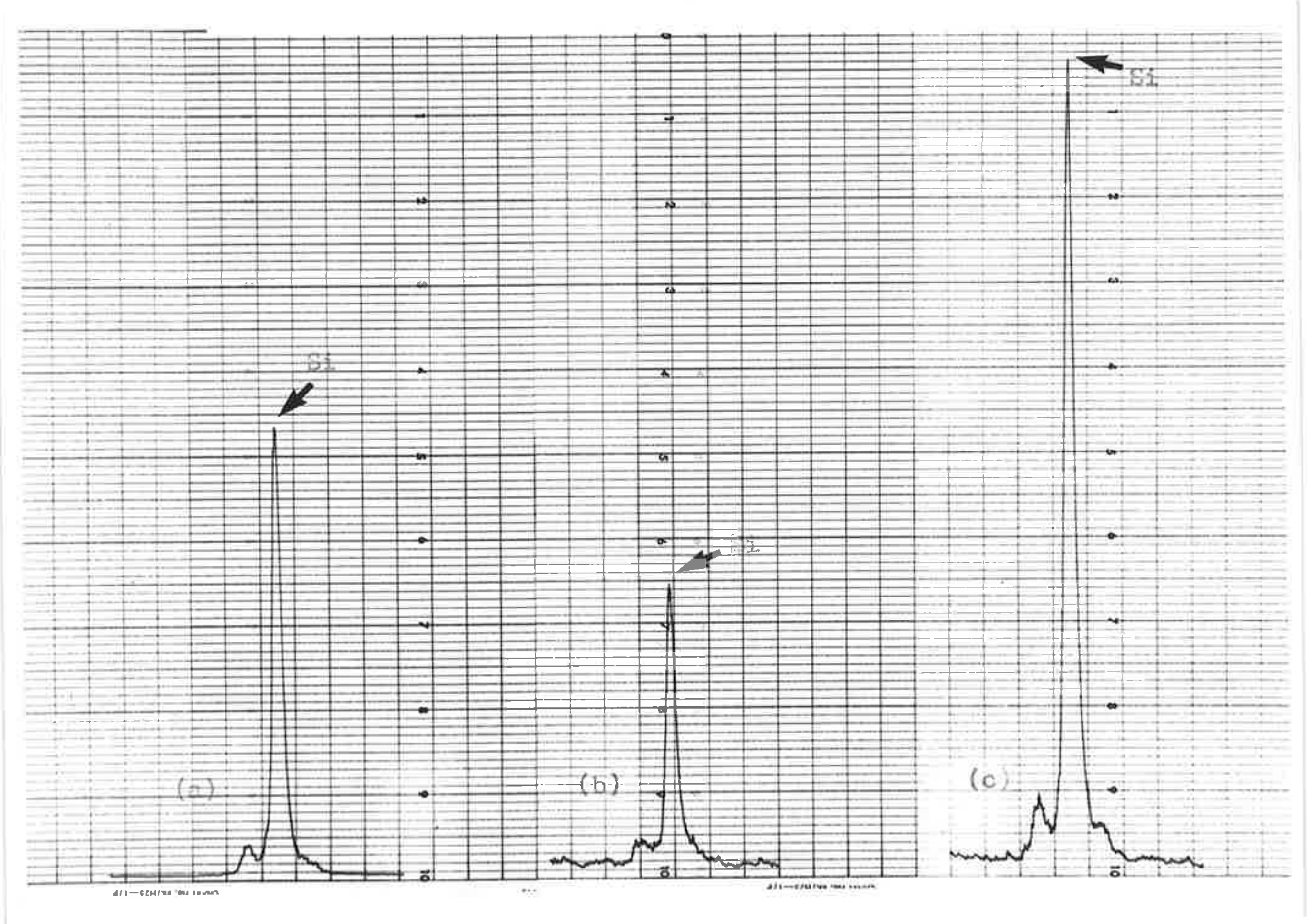


Figure 34: Composite of three energy spectra of (a) pure silicone filings in the rat and of the refractile particles in liver samples from (b) Autopsy Case 22 and (c) Autopsy Case 11. All three spectra show a high peak for silicon (arrow). Note the similarity of the liver material to the silicone filings (See Appendix IIb for scanning details).

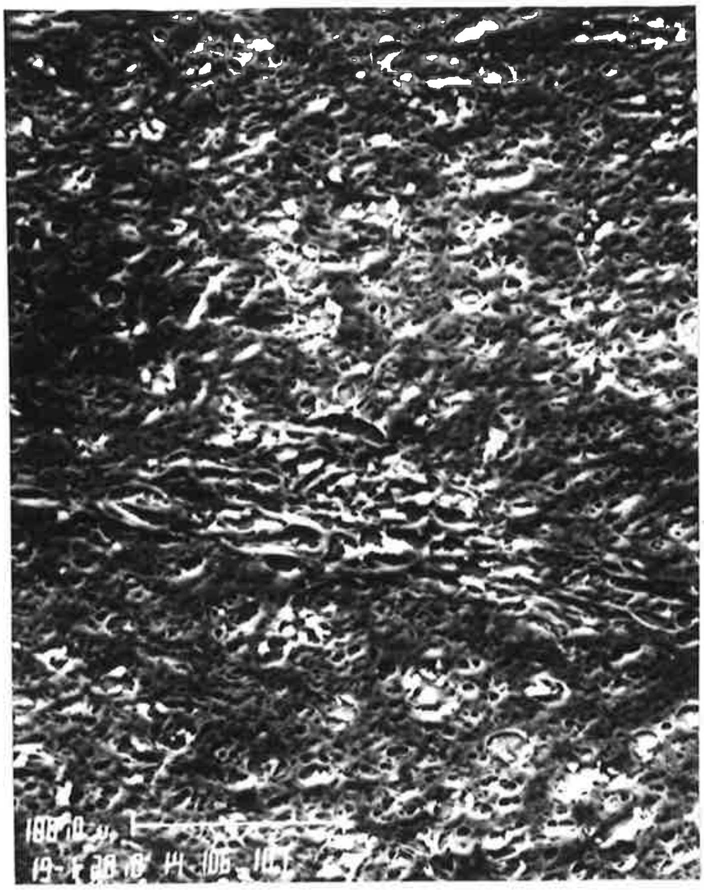


Figure 35a: Scanning electron micrograph of the cut surface of the liver showing a focus of silicone deposition (Autopsy Case 22 - photograph courtesy of Prof J L Abraham).

Figure 35b: Scanning electron micrograph of the same area as Figure 35a using the back-scattered electron mode. The darker areas show deposits of silicone (photograph courtesy of Prof Abraham)

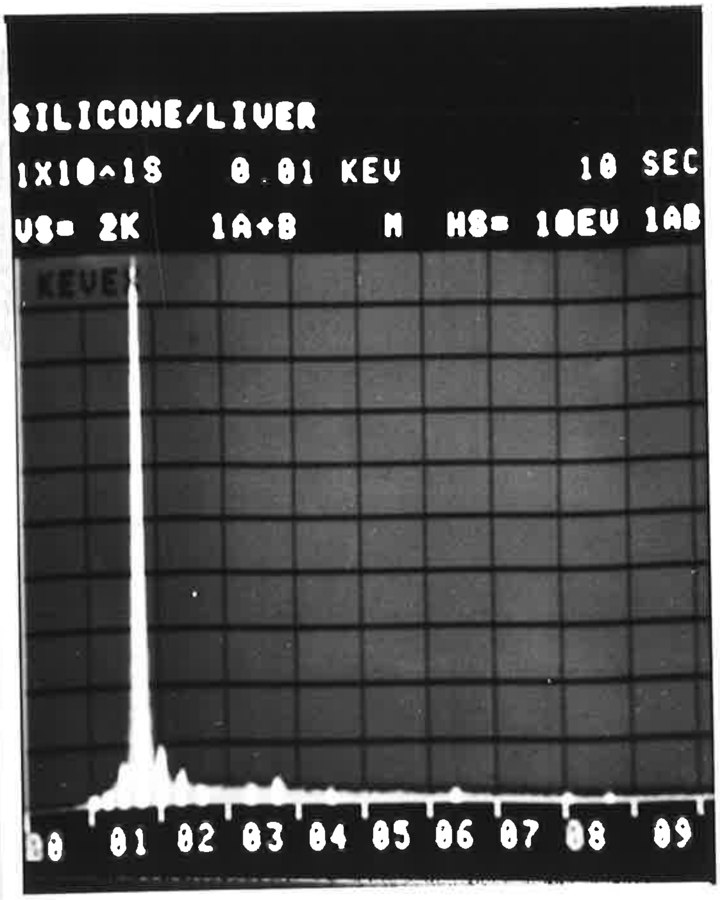


Figure 35c: Spectrum from an energy-dispersive X-ray analysis of the silicone deposits in Autopsy Case 22. The large peak at 1.74 kev identifies silicon. (Photograph kindly provided by Professor J L Abraham, University of California).

VII.c. ATOMIC ABSORPTION SPECTROMETRY ANALYSIS OF THE FOREIGN MATERIAL

The analytical chemistry of the silicones abounds with problems which have not yet been satisfactory solved. Noll (1968) reports that the determination of the constitution of relatively complex polymeric silicones is a problem which can be solved only by the use of various methods, and often not unambiguously. "The desire of determining small concentrations of silicones in solid or liquid technical products cannot always be satisfied". The literature reveals only three reports in which silicone concentrations were quantitated in biological material. Solomons and Jones (1975) presented a detailed study of the quantitation of silicone (polydimethylsiloxane) in the tissues of a woman who died following injections of silicone into her breasts. These authors utilised the method of atomic absorption spectrometry in their analysis. Another two reports of fatal and near fatal episodes following inadvertent intravascular injections of silicone during breast augmentation mentions atomic emission spectrography (Ellenbogen et at, 1975) and mass spectrometry (Uretsky et al, 1979) as methods of analysis. In both these reports analysis was performed by commercial agencies. Correspondence with one of the authors disclosed the address of one of these agencies but financial constraints prevented further follow-up (Appendix IId). Noll (1968) discussed other methods of analysis and infrared and ultraviolet spectrometry have been used in experimental studies (Gayou, 1976; Bergman & van der Ende, 1979).

Facilities for quantitative analysis of silicone were eventually found. Mr J Waters of the Analytical Chemistry Division of the Australian Mineral Development Laboratories performed atomic absorption spectrometry analysis of wet tissues which were available from 11 postmortems (Appendix IIe and IIf). Analysis was performed in a Varian-Techtron Atomic Absorption Spectrometer (model AA775) with a graphite furnace atomization (model CRA-90) and Amdel filament-in-furnace atomization (model FIFA-3). Samples of liver, spleen, lung, lymph node and perinodal tissue from cases shown microscopically to contain silicone particles as well as from controls were examined. The results expressed as chloroform-soluble polydimethylsiloxane are shown in Table V. In the cases where samples of spleen were available for analysis, they were found to contain as much as twice the amount of silicone per unit weight of wet tissue when compared to liver samples.

Table V: Atomic Absorption Spectrometry Analysis of 11 Autopsies of Haemodialysed Patients with Silicone Embolisation

	* S I L I C O N E (ug/g wet tissue)			
Case No.	Liver	Spleen	Lymph Node	Lung
1	20	NA	NA	NA
4	15	NA	NA	NA
11	20	NA	NA	NA
16	15	70	50	15
20	30	80	NA	NA
21	45	NA	NA	NA
22	160	NA	NA	NA
Control 1	10			
Control 2	15			
Control 3	10			
Control 4	10			

*Silicone expressed as chloroform-soluble polydimethylsiloxane

NA = wet tissue not available for analysis

Control 1 = non-renal patient

Control 2 = haemodialysed patient with no detectable silicone by phase contrast microscopy

Controls 3 & 4 = peritoneal dialysis only patients

VIII. CLINICO-PATHOLOGICAL CORRELATION

VIII.a. ANALYSIS OF CLINICAL DATA

VIII.b. STATISTICAL ANALYSIS OF HISTOLOGIC QUANTITATIVE STUDIES

VIII.c. EXAMINATION OF SEQUENTIAL BIOPSIES

VIII. CLINICO-PATHOLOGICAL CORRELATION

VIII.a. ANALYSIS OF CLINICAL DATA

The case notes of all patients who had accumulated silicone in their tissues were reviewed for the following data: 1) duration of haemodialysis, 2) highest level of serum aspartic transaminase (AST) measured, 3) duration of continuously raised AST, 4) hepatitis B antigenemia and 5) serum titres against cytomegalovirus, toxoplasma, and herpes simplex virus.

Although full biochemical liver function tests were available in all cases, the serum AST level was chosen as the most reliable index of hepatocellular necrosis (Burke, 1975). "Inflammation of the liver continuing without improvement for at least six months", an international definition of chronic hepatitis (U S Government Printing Office, 1976) was adopted for this study. Hepatic inflammation was assessed by serum AST estimations (normal 0-50 IU/L). These data are presented in Tables VIa and VIb. The duration of haemodialysis associated with microscopically detectable hepatic silicone ranged from 2-84 months (mean 24 months). The patient with the shortest duration of haemodialysis also had hepatic metastases from an adenocarcinoma of the colon (Autopsy Case 1). Barely detectable but definite quantities of silicone were observed in this case. The patient with the longest duration of exposure also showed the largest quantity of silicone in his tissues. In the liver, this accumulation was associated with a grade 4 fibrosis.

Of the 18 patients who had liver biopsies which contained silicone, 17 had levels of serum AST which exceeded 50 IU/L. The only case with normal AST levels was biopsied because of seropositivity for HBsAg. In 10 of these cases, the AST levels were persistently raised beyond six months (chronic hepatitis), three of these having biopsies which revealed a granulomatous inflammation. Similarly, chronic

Table VIa: Clinical and Histologic Features of Cases with Silicone in their Livers: 18 Biopsy Cases

Case No	Duration of Haemodialysis (months)	Silicone Score	Grade of Fibrosis	Grade of Inflamm.	Maximum AST (IU/L)	Duration of Raised AST (months)
1	2	1	2	1	500	<6
2+	2	1	1	1	N	-
3	6	1	2	2	110	<6
4	6	2	2	2	240	<6
5	8	1	2	2	300	12
6+	11	1	3	4	>700	<6
7+	12	1	1	1	160	8
8	15	2	1	1	400	15
9	15	3	3	4*	130	7
10	17	2	3	1	170	<6
11	17	2	2	1	170	12
12	18	2	1	2	250	7
13	20	2	1	0	> 350	< 6
14	28	3	2	3*	350	15
15	30	3	2	1	160	7
16+	39	2	2	2	200	< 6
17	52	4	3	4*	700	10
18	54	3	3	2	180	6½

+ = HBsAg positive cases, *= granulomatous inflammation
silicone score: 1=barely visible, 2=readily visible, 3=clumped aggregates, 4=confluent clumps
fibrosis grading: 1=expansion of portal tracts by fibrosis, 2=stellate scars in portal areas, 3=linkage of stellate scars, 4=encircling of nodules of hepatocytes by fibrous bands
inflammation grading: 0=absent, 1=mild, 2=moderate without giant cells or granulomata, 3=moderate with giant cells or granulomata, 4=severe with or without giant cells or granulomata

Table VIb: Clinical and Histologic Features of Cases with Silicone in their Livers: 22 Autopsy Cases

Case No	Duration of Haemodialysis (months)	Silicone Score	Grade of Fibrosis	Grade of Inflamm.	Maximum AST (IU/L)	Duration of Raised AST (months)
1	2	1	2	1	500	<6
2	6	1	2	2	60	9
3	7	1	2	1	N	-
4	7	3	2	2	N	-
5	9	1	1	0	N	-
6	9	4	2	3*	100	<6
7	10	1	1	0	N	-
8	10	1	1	0	N	-
9	13	1	4	2	320	<6
10	18	2	3	1	N	-
11	27	3	3	4*	130	7
12	28	3	2	3*	350	15
13	28	3	2	3*	200	<6
14	31	3	2	1	N	-
15	36	3	2	3*	>900	<6
16	38	2	1	3*	N	-
17	42	4	2	3*	230	<6
18	43	3	1	3*	220	<6
19	51	2	2	1	300	<6
20	54	3	4	3*	350	<6
21+	69	3	3	2	200	<6
22	84	4	4	2	>250	7

+ = HBsAg positive cases, * = giant cells or granulomata
silicone score: 1=barely visible, 2=readily visible, 3=clumped aggregates, 4=confluent clumps
fibrosis grading: 1=expansion of portal tracts by fibrosis, 2=stellate scars, 3=linkage of stellate scars, 4=encircling of hepatocyte nodules by fibrous bands
inflammation grading: o=absent, 1=mild, 2=moderate without giant cells or granulomata, 3=moderate with giant cells or granulomata, 4=severe with or without giant cells or granulomata

hepatitis was observed in four of the 14 autopsy cases which had raised serum AST. Three of these had previously been biopsied. In all autopsy cases the AST levels tended to fluctuate and only two patients died with raised levels of this enzyme, both being cases of chronic granulomatous hepatitis.

Four patients were positive for HBsAg (Biopsy Case 2,6,7,16. Case 16 came to autopsy). A raised titre to cytomegalovirus was found in four patients (Biopsy Case 11, 13, 15 and Autopsy Case 9), and rising titres to herpes simplex virus was detected in two patients (Biopsy Case 13 and Autopsy Case 9).

VIII.b. STATISTICAL ANALYSIS OF HISTOLOGIC QUANTITATION STUDIES

The quantitative scores assigned to the amount of silicone, and the severity of inflammation and fibrosis in the liver allowed statistical correlation of these variables. Employing Kendall's non-parametric statistical method (Hollander & Wolfe, 1973), a positive correlation was found between the amount of silicone in the liver and the duration of haemodialysis ($K = 150$, $p < 0.0001$). A significant correlation was found between the amount of silicone and the grade of inflammation ($K = 198$, $p = 0.002$) and between the amount of silicone and the severity of fibrosis ($K = 150$, $p = 0.02$) (Appendix III). Cases serologically positive for HBsAg, a case of micronodular cirrhosis related to alcohol (Autopsy Case 9) and a case with metastatic carcinoma (Autopsy Case 1) were excluded from statistical analysis because these conditions may be associated with inflammation and fibrosis per se.

VIII.c. EXAMINATION OF SEQUENTIAL LIVER BIOPSIES

In nine patients more than one liver sample was available for examination. In six of these the time interval between obtaining the liver samples was sufficiently long to allow examination for any progression of the pathology (Table VII). In Autopsy Case 6, a liver biopsy taken before commencement of haemodialysis showed no silicone or any significant morphologic alteration. At post-mortem 37 months later, after 9 months of haemodialysis, a granulomatous hepatitis was present with abundant particles of silicone. Autopsy Case 21 showed barely visible particles of silicone after three months of dialysis whereas silicone was readily seen after 33 months of dialysis. At autopsy after a total of 69 months of exposure, silicone occurred in prominent clumps and inflammation was of a moderate severity and linking scars were observed in the liver (Figure 36). Autopsy Case 22 showed no detectable silicone after 10 months of dialysis, but, at postmortem after a further 74 months of repeated dialysis, he had developed grade 4 silicone and grade 4 fibrosis in the liver (Figure 37).

Some general remarks could be made from the changes seen in the few cases in which serial liver samples were available. It was clearly demonstrated that silicone was not present prior to haemodialysis and the quantity of hepatic silicone tended to increase with the duration of exposure.

Table VII: CASES WITH SEQUENTIAL LIVER SAMPLES

Case No.	Duration of Dialysis	Silicone	Fibrosis	Inflammation
A.1	2 months	1	2	1
	2¼ months	1	2	1
A.6	0 months	0	0	0
	9 months	4	2	3*
A.10	17 months	2	3	1
	19 months	2	3	1
A.11	25 months	3	3	4*
	27 months	3	3	4*
A.12	0 months	0	0	1
	28 months	3	2	3*
	28 months	3	2	3*
A.21	3 months	1	1	1
	33 months	2	2	2
	69 months	3	3	2
A.22	0 months	0	0	0
	84 months	4	4	2
B.12	3 months	0	0	1
	18 months	2	1	2
B.15	22 months	3	2	1
	30 months	3	2	1

* = with giant cells or granuloma formation

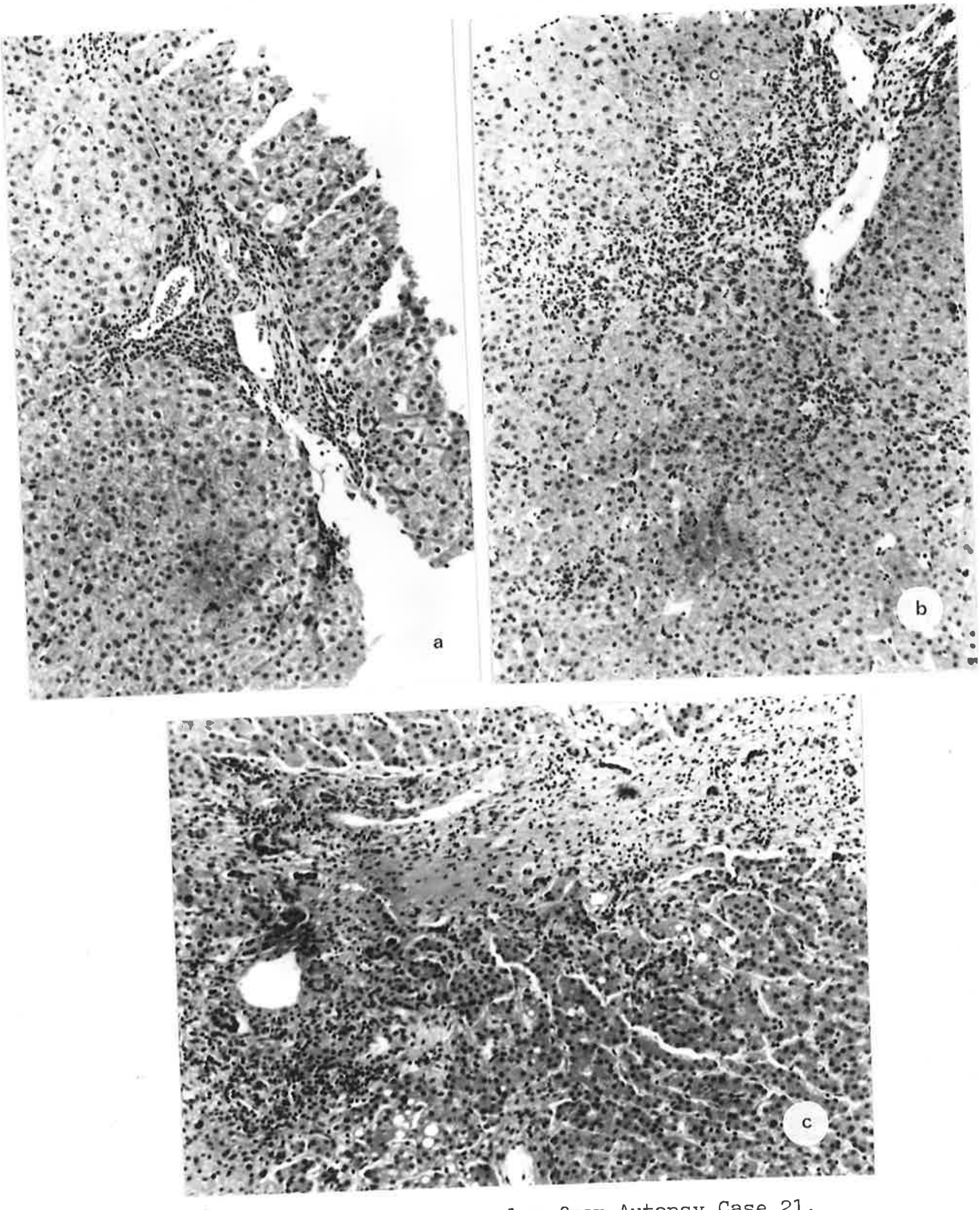


Figure 36: Sequential liver samples from Autopsy Case 21. (a) after 3 months dialysis, silicone 1, fibrosis 1, inflammation 1, (b) after 33 months dialysis, silicone 2, fibrosis 2, inflammation 2, (c) after 69 months dialysis, silicone 3, fibrosis 3, inflammation 2 (H&E, x 125).

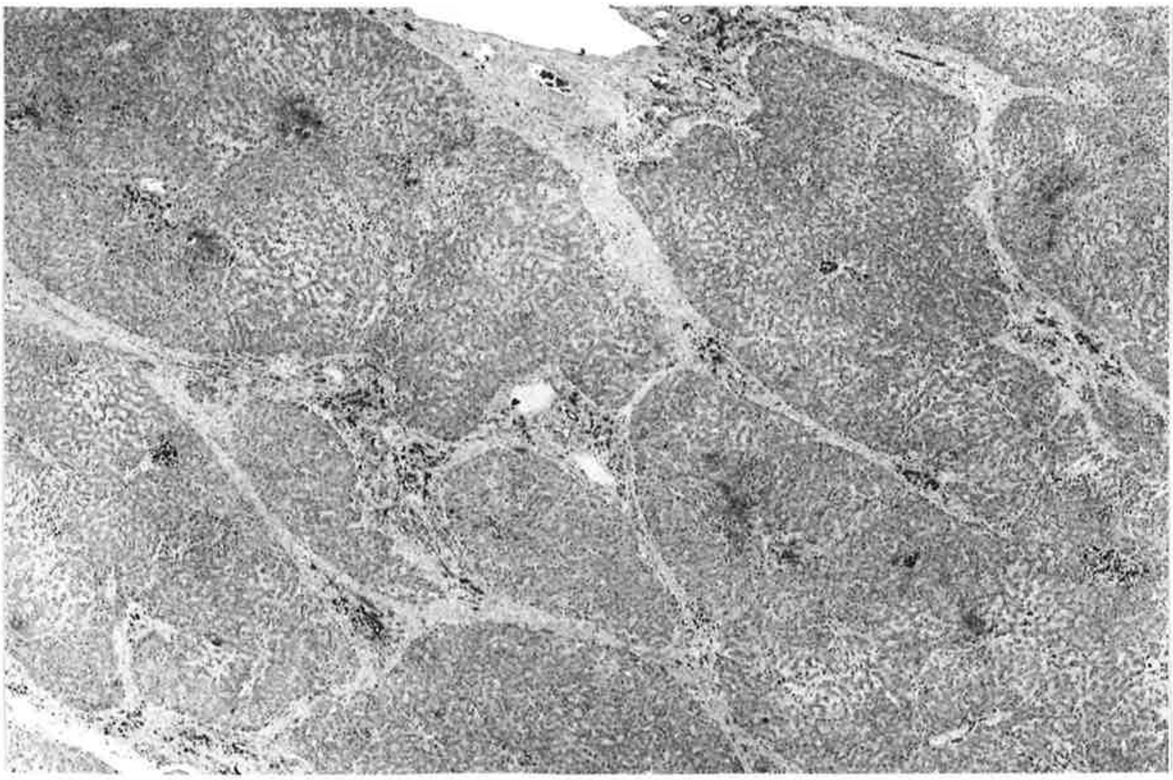


Figure 37: Liver after 84 months of haemodialysis. There is marked fibrosis which encircles nodules of hepatocytes. Central veins, however, remain preserved in most of these nodules and the fibrous linkage is between portal areas. Macrophages containing abundant particles of silicone are also present in the portal areas and fibrous scars (H & E, x 100).

IX. IN VITRO SPALLATION TESTS

IX.a. ATOMIC ABSORPTION SPECTROMETRY

IX.b. SCANNING ELECTRON MICROSCOPY

IX. IN VITRO SPALLATION TESTS

The word "spall" is defined in the Shorter Oxford Dictionary as "to break (ore) into smaller pieces" or "to split or break off" or "to chip or splinter" (Little et al, 1963). The word "spallation" has been introduced for "the release of small plastic fragments into the body" (FDA Medical Standards Publication, 1980). The possibility of spallation occurring from blood lines has been recognised and because of its potential hazards, Kjellstrand (1978) has urged that "spallation tests be performed on all blood tubings, filters and pumps before their clinical use".

IX.a. ATOMIC ABSORPTION SPECTROMETRY ANALYSIS

In vitro testing was set up to prove the source of silicone contamination in our cases. Three separate experiments were performed. In the first, haemodialysis blood lines (Tuta Laboratories Australia, Pty, Ltd) were connected in a continuous circuit to a plastic bag containing 500 ml of whole blood (Figure 38). The 40-cm segment of silicone tubing was placed in a "just occlusive" roller pump. The pump produced a compression of about 20-30% of the tubing diameter and flow rates were set at 250-300 ml per minute to simulate dialysis. Blood samples of 20 ml portions were drawn in a disposal plastic syringe (Terumo Australia, Pty, Ltd) at the commencement of the experiment and subsequently after 5, 20, 48 and 96 hours of pumping. The samples were stored in sterile glass containers with metal tops.

A second experiment was performed in a similar fashion with a new

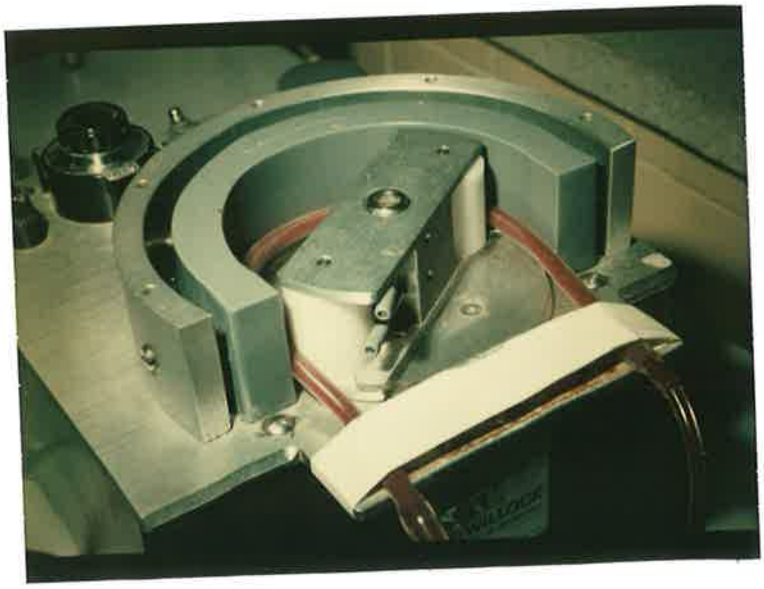


Figure 38: In vitro testing of silicone tubing. Blood from a plastic bag is continuously circulated through new blood lines and the 40-cm length of silicone tubing is located in the roller pump as shown in the close-up photograph. Flow rates of 250-300 ml/min simulate dialysis and the rollers compress the tubing to 20-30% of its diameter.

set of blood lines, but this time no blood samples were taken. At the completion of 20 hours of pumping, the silicone tube was rinsed in water and prepared for examination.

In the third experiment a similar procedure was repeated on a new set of blood tubings. Five hundred millilitres of whole blood were continuously pumped through the silicone tubing for a period of five hours which represents the average duration of use during haemodialysis. At the completion of this period of pumping the silicone tubing was rinsed in water and prepared for examination.

All blood samples obtained during the first experiment were submitted for analysis by atomic absorption spectrometry and the levels of silicone detected are presented in Figure 39 (Appendix IIf).

A new unused silicone tubing and the tubings which were subjected to five and twenty hours of pumping were examined with a dissecting microscope. Deep slashes were found in three locations on the external surface of the 20-hour tubing but the other tubings were grossly unaltered. All tubings were divided longitudinally and their internal surfaces examined with a dissecting microscope. The new tubing displayed a rough internal surface with a vague basket-weave pattern (Figure 40a). After five hours of use there was an accentuation of this pattern as if the tube had been stretched. Broad anastomosing grooves were present between elevated areas or ridges which generally ran along the circumference of the tubing (Figure 40b). In addition, pit-like depressions could be seen on the ridges. After 20 hours of pumping, the elevated ridges appeared to have been flattened as they were broader while the intervening grooves were narrower (Figure 40c) Pitting and scooped-out defects were more evident (Leong & Gove, 1982).

IX.b. SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) is an established method of examining surfaces of biological and non-biological material

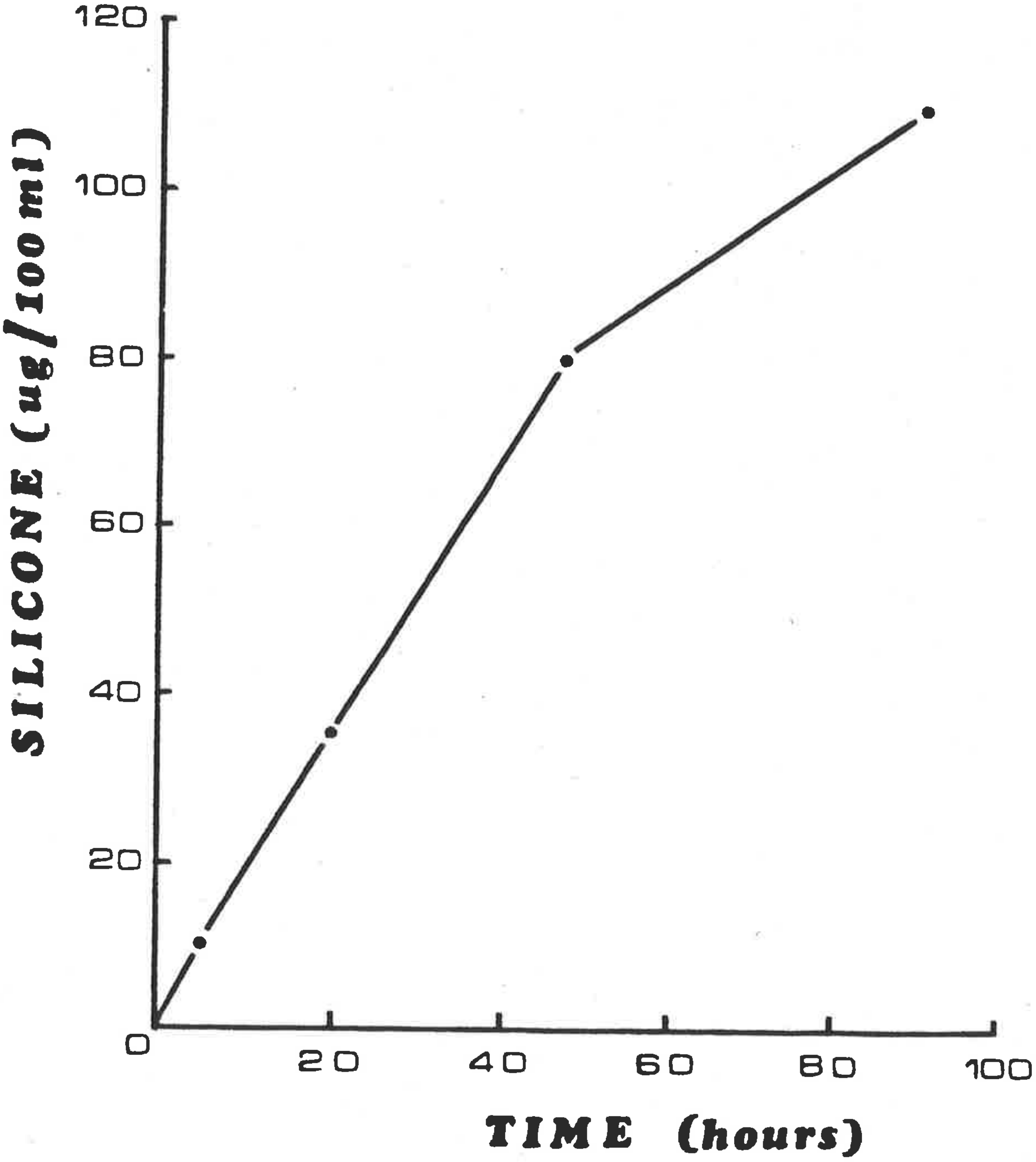


Figure 39: Spallation tests on silicone tubing. Concentrations of silicone as chloroform-soluble polydimethylsiloxane in whole blood during continuous pumping at 250-300 ml/min flow rate. Silicone measured by atomic absorbtion spectrometry.

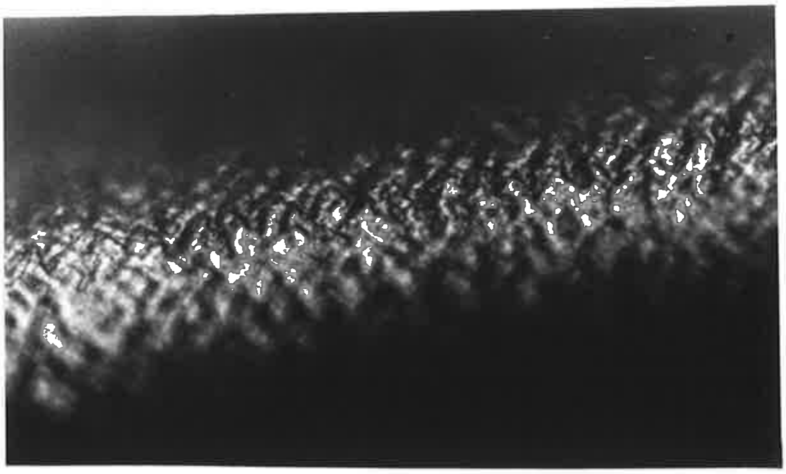


Figure 40a: New silicone tubing, internal surface. Note the intrinsic reticular pattern (x 10).

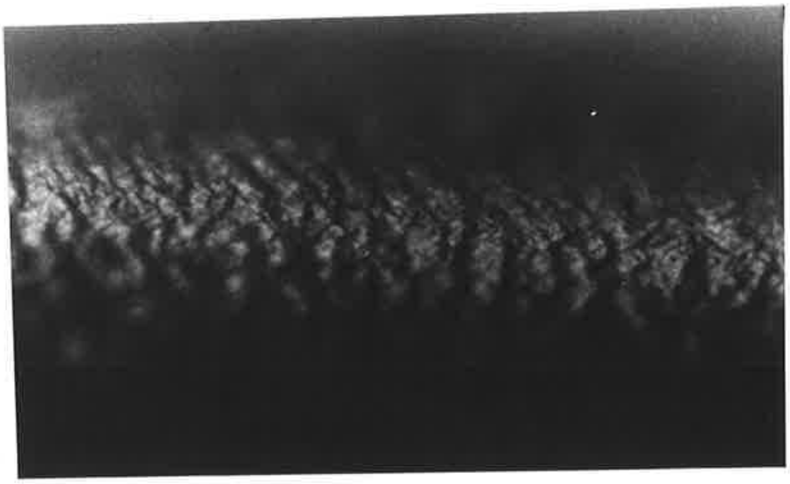


Figure 40b: Silicone tubing after 5 hours pumping. Broad grooves are present between ridges producing a somewhat eccentuated pattern compared to the unused tubing. Small pits are also visible on the ridges (x 10).

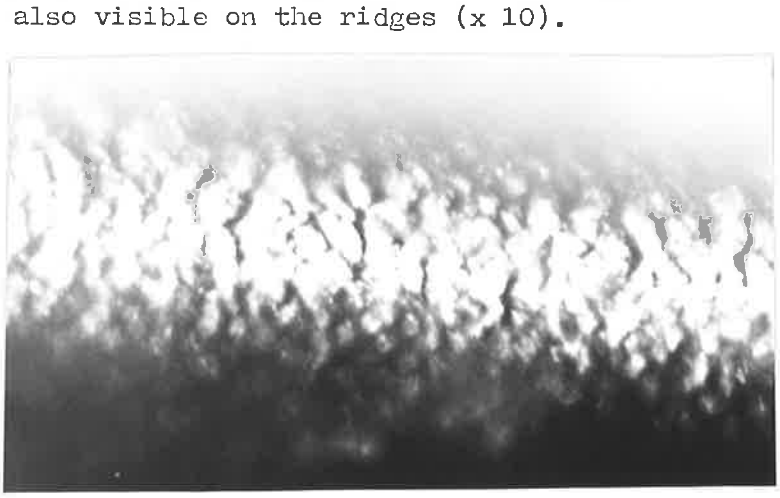


Figure 40c: Silicone tubing after 20 hours of pumping. The ridges are broader and the grooves narrower, suggesting a compressive effect. More pits on the ridges are evident (x 10).

(Leong et al, 1975). Three areas were sampled from each of the unused, five-hour and 20-hour tubing. Each sample consisted of a 1 cm length of a longitudinal half of the tubing. They were mounted on SEM stubs with their internal surface uppermost and coated with a 10 nm layer of gold-palladium and examined in a JEOL model JSM-T20 scanning electron microscope.

The reticular recurring pattern of the unused tubing as revealed by the dissecting microscope was confirmed (Figure 41). After five hours of pumping there was patchy loss of this intrinsic pattern. In areas, the surface appeared smoothened-out and prominent superficial splits and tears were visible (Figure 42). The flaking edges of these defects had irregular crinkled outlines very similar to those of the embolised particles in the liver and other tissues (Figure 15). The 20-hour specimen revealed a generalised loss of the intrinsic irregularity of the tubing. The internal surface was diffusely smooth and showed gentle undulations with scattered, large, smooth-edged, sloping depressions. In addition to occasional splits, there were also striking punched-out holes and gouged-out cavities (Figure 43).

Both tubings which had been subjected to pumping showed fibrin and erythrocytes adherent to their internal surfaces. Occasional patches of the tubing were covered by bacterial growth. Prominent pieces of solid material were also seen on the surface but these may have been related to dirt and fragments released during the cutting of the tubing.

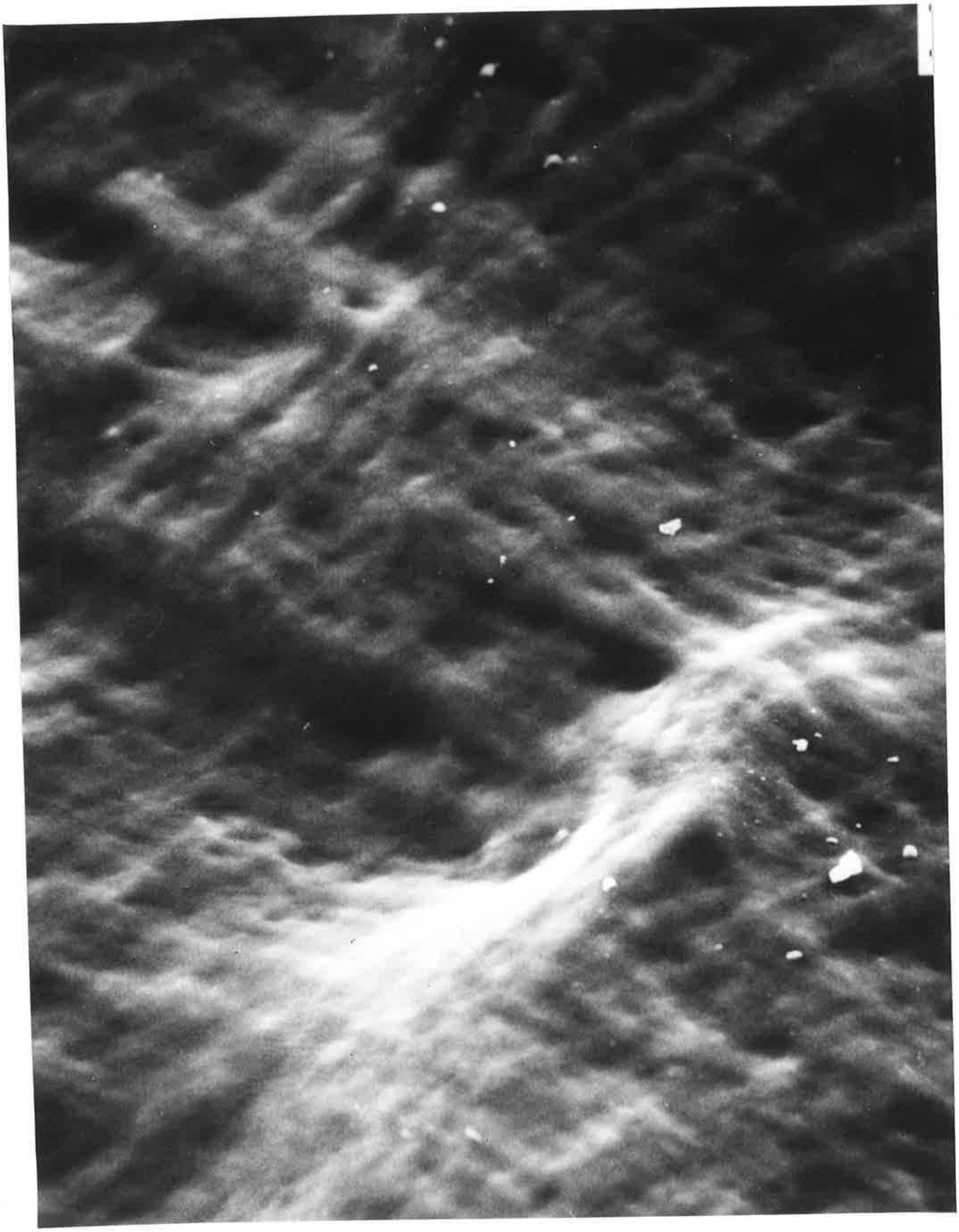


Figure 41: Scanning electron micrograph of the internal surface of a new silicone tubing. An intrinsic reticular or basket-weave pattern is evident (x 1,000).

Figure 42: Scanning electron micrograph of the internal wall of the silicone tubing after 5 hours of pumping. There is focal smoothening-out of the intrinsic basket-weave pattern and prominent splits are seen on the surface. The flaking edges of these splits show an irregular outline. Note the solid debris and fibrin on the surface (x 1000).

Figure 43: Silicone tubing after 20 hours of use. Besides the loss of intrinsic pattern, splits and tears are seen in the generally smooth undulating surface. Also present are prominent holes and gouged-out cavities (x 1000).

X. DISCUSSION

X.a. THE CHEMISTRY OF SILICONE

X.b. EXPERIMENTAL TOXICITY OF SILICONE

X.c. MEDICAL USAGE OF SILICONE

X.d. CLINICAL COMPLICATIONS OF SILICONE USAGE

X.e. THE PATHOLOGY OF SILICONE MIGRATION IN HAEMODIALYSIS PATIENTS

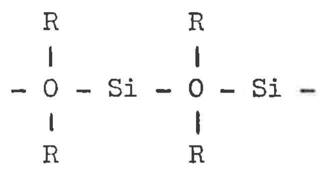
X.f. SEQUEL TO THE IDENTIFICATION OF SILICONE-INDUCED CHRONIC HEPATITIS

X.g. CONCLUSIONS

X. DISCUSSION

X.a. THE CHEMISTRY OF SILICONE

Polymeric silicone used for medical purposes consists primarily of polydimethylsiloxane and occasionally polydiphenylsiloxane. This polymer is composed of repeating units of



where R is an organic radical such as CH_3. The name "silicone" was coined at a time when it was thought that far-reaching analogies to the chemistry of carbon could be found in the chemistry of silicon. The term was adopted by analogy with "ketone", since the structural unit of the chain shown above, R_2SiO, appeared to correspond to a ketone, R_2CO. The analogy goes little further than this as their chemical properties are quite contrasting (Noll, 1968). "Polyorganosiloxane" is a more precise term for these compounds than "silicone". The name "siloxane" was based on the formulation of Si-O-Si unit as sil-oxane, and this has found general acceptance in scientific nomenclature (Noll, 1968). The name "silicone", however continues to be widely used. For reasons of simplicity of speech it has become well-entrenched and is difficult to abandon.

Polymeric silicone, depending on the extent of cross linking involved and the curing method used, can be manufactured as a fluid, solid or gel. However, there are no essential chemical nor presumably biological differences between these various physical forms (Arkles, 1981).

X.b. EXPERIMENTAL TOXICITY OF SILICONE

Rees et al (1967) and Ben-Hur et al (1967) performed some of the earlier biological studies on silicone. Subcutaneous injections of liquid silicone in rats produced a round cell infiltrate composed of lymphocytes and macrophages. This mild inflammation lasted up to six months. At 12 months there were increased amounts of collagen in the area and an occasional lymphocyte and giant cell were found lying adjacent to a silicone-filled cyst. Subcutaneous administration of massive amounts of silicone, however, produced a severe inflammatory reaction and adipose cells in the immediate vicinity showed varying degrees of atrophy with the cytoplasm of these fat cells containing small regular vacuoles of silicone. Macrophages with phagocytosed colourless silicone fluid were also observed. Massive amounts of subcutaneous silicone also resulted in widespread visceral dissemination of the substance (Rees et al, 1967). There was atrophy of abdominal and epicardial adipose tissue and many abdominal organs including adrenals, lymph nodes, liver, kidney, spleen, pancreas and overies showed focal silicone granuloma-like lesions (Ben-Hur et al, 1967). Similar reactions were seen with intraperitoneal injections of the material.

Ashley et al (1967) reviewed some of the earlier animal experimentation with fluid silicone. Oral ingestion of silicone by rats and humans was reported not to produce any discernible effects. Intravenous injections of silicone in dogs appeared to simulate intravenous air injections. Small quantities resulted in systemic distributions whereas large doses produced emboli and death (Ashley et al, 1967). A much smaller dose of silicone proved fatal when injected into the arterial system.

Andrew (1966) showed that clear cytoplasmic vacuoles were present in neutrophils and mononuclear cells in the vicinity of subcutaneous deposits of fluid silicone. According to him, these circulating cells could phagocytose silicone fluid. Rees et al (1967) have also observed similar vacuoles in leukocytes taken from the peripheral blood of mice and baboons after subcutaneous and intra-peritoneal injections of silicone. They also noted a similar finding in the red blood cells of rats and suggested that silicone fluid may have some effect on the haemopoietic system.

Reports of malignancy in mice following cutaneous administration of silicone (Ben-Hur &Neuman, 1963; 1965) have been attributed to the high incidence of spontaneous adenocarcinoma in these animals (Grasso et al, 1964). A definite relationship between carcinogenesis or sarcogenesis and the administration of silicone fluid has not been demonstrated (Rees et al, 1970).

X.c. MEDICAL USAGE OF SILICONE

Few chemical compounds are capable of so many widespread applications as the silicones. They are used in engineering in the construction of machines and apparatus as well as for electrical insulation. Silicone oils and greases serve as heat-transfer media and as lubricants. Silicones also play the part of auxiliary in various branches of industry. As lacquers they are used for the surface protection of metals, as mold-release agents in the rubber and plastics industry, as water repellents in the textile industry; in the protection of masonry, in the glass industry, and their defoaming properties are employed in the manufacture of various paints.

Their stability and physiological inertness have resulted in extensive applications in medicine, pharmacy and cosmetics. Because of their hydrophobic properties silicone oils are added to agents for skin care, nail varnishes, lipsticks, anti-sunburn preparations and even to toothpastes, performing in each case, the function of a water-repellent agent (Noll, 1968). Many dermatologically active materials can be incorporated in silicone-containing ointment bases to give stable compositions. In the medical field, silicone ointments or pastes protect skin endangered by continuous wetting as in the perineal area of babies, around colostomy openings and in wounds healing by secondary intention.

Artificial conduits are treated with silicone to induce flexibility and low permeability. Artificial arteries, tubes and drains are made of silicone rubber and as permanent implants silicone catheters are said to evoke no tissue response. Blood clotting is retarded at surfaces of silicone products or on walls of vessels coated with silicone films (Noll, 1968); hence, silicone rubber tubes are used for blood transfusions, and glass vessels and syringes are silicone-coated.

Silicone fluid was once used extensively in corrective and cosmetic surgery but due to disastrous adverse consequences (Murray, 1967; Vinnik, 1976; Pearl et al, 1978; McDowell, 1978) its use is now significantly restricted. Solid elastomers, on the other hand, are used extensively as implant material. They are used in ear, nose, chin and penile prostheses, in orthopaedic implants and as heart valve components, and special invasive tubings. A newer generation of solid silicone copolymers is currently being used for contact lenses. Silicone in a gel state is mainly used in implants such as mammary prostheses and testicular prostheses. Approximately 75,000 mammary prostheses and 4,000 testicular prostheses are implanted each year in the United States of America, most of these operations being cosmetic rather than reconstructive (Mishra, 1981).

X.d. CLINICAL COMPLICATIONS OF SILICONE USAGE

Although the various forms of solid silicones are considered to be safe because of their excellent haemocompatibility, experimental studies have shown that definite physical changes do occur as a result of ageing in all types of implanted silicone rubbers (Roggendorf, 1976). Recent clinical reports confirm that deterioration and degradation of silicone devices can occur with loss of material strength and subsequent fracture of the retrieved implant. Physiochemical decay of orthopaedic prostheses has been shown to produce a giant cell reaction in the adjacent synovium and migration to regional lymph nodes has resulted in a granulomatous response (Aptekar et al, 1974; Christie et al, 1977; Kircher, 1980).

Despite its relative physiological inertness, there is ample experimental as well as clinical evidence to show that silicone can evoke a foreign body granulomatous inflammation. The well-recognised "silicone mastitis" (Symmers, 1968) is the result of a chronic granulomatous response to injections of silicone or to the "bleed" or leakage from silicone breast implants (Smahel, 1979; Bergman & van der Ende, 1979). In vitro studies have demonstrated that exudation of silicone can occur through structurally intact envelopes of gel-filled breast prostheses (Bergman & van der Ende, 1979).

Silicone is fibrogenic and stimulates the formation of a fibrous capsule around the prosthesis and injection site. Aside from its presence in pericapsular macrophages, silicone has been identified in the lymphatic system draining the implant region as well as within macrophages and multinucleated giant cells inside capillaries and small vessels around the implants (Jenny & Smahel, 1979). The migration to regional lymph nodes produces a granulomatous lymph-adenitis with refractile vacuoles in giant cells (Hausner, 1981).

Facial injections of silicone have produced skin discolouration, induration and subcutaneous nodule formation. Subcutaneous injection of the substance with excessive force has produced blindness (Ashley et al, 1967). Illicit injections of silicone fluid for breast augmentation has resulted in an acute febrile illness which on occasions has been fatal (Solomons & Jones, 1975; Ellenbogen et al, 1975; Celli et al, 1978). Ellenbogen et al (1975) reported four patients who developed adverse reactions to subcutaneous injections of silicone. A granulomatous hepatitis was found in three cases and this was associated with raised levels of glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, lactic dehydrogenase and alkaline phosphatase in the blood of two patients. These enzymes were marginally elevated in the third while the fourth patient died a sudden death with evidence of widespread dissemination of silicone in many organs. Another report describes a case where bilateral implants resulted in high fever, diffuse arthritis, renal failure and bilateral pulmonary infiltrates (Uretsky et al, 1979). Dramatic improvement occurred with removal of the prostheses and silicone was identified in the blood, urine and sputum.

Silicone rubber was extensively used in the manufacture of cardiac prostheses before degeneration of the silicone ball (ball variance) became recognised as a common cause of late failure of the ball-valve prosthesis. Many reports described the degeneration and fracture of these silicone rubber balls with migration of macroscopic ball fragments into the aorta and large vessels (Roberts & Morrow, 1968; Hameed et al, 1968; Cohen et al, 1968; Fiegenberg et al, 1969). Microscopic granulomata were found commonly in the liver and spleen and also in the rest of the mononuclear phagocytic system (Ridolfi & Hutchins, 1974). Prominent refractile particles were seen engulfed by macrophages and giant cells. A postmortem liver biopsy study was performed on three patients with aortic ball variance who showed disseminated poppet material in the liver, spleen and, to a lesser extent, in the remaining mononuclear phagocytic system (Ridolfi & Hutchins, 1974). Five per cutaneous needle biopsies were performed on each cadaver and the specimens obtained were routinely processed and serial sections were examined by light microscopy. Granulomas containing refractile particles were readily identified in the portal triads and hepatic sinusoids in all biopsies. The ability to detect poppet material in liver biopsy specimens in the face of the diagnostic difficulties encountered in patients with ball variance prompted these authors to make the interesting suggestion that liver biopsy may be the best means available to detect prosthetic valve ball variance.

Another area of cardiac surgery where silicone is employed is in the bubble oxygenator of the cardiac bypass machine. The anti-foaming properties of silicone are utilised in a de-foaming chamber so that gas embolism does not occur. Frick et al (1974) reported that in 40% of the patients who died after extracorporeal circulation, organ emboli of the anti-foaming material could be demonstrated. They were able to show by SEM that particles of silicone could be dislodged by streaming of blood in the chamber, these emboli being carried into the capillaries of the brain and kidney where they produced infarcts.

X.e. THE PATHOLOGY OF SILICONE MIGRATION IN HAEMODIALYSIS PATIENTS

Despite its relative physiologic inertness, there is ample experimental evidence to show that silicone can stimulate a mild inflammatory response which persists up to six months. After 12 months a few lymphocytes and giant cells remain around a thick fibrous capsule which forms around the subcutaneous deposit of silicone (Rees et al, 1967). The fibrogenic and inflammatory properties of silicone are similarly demonstrated in humans where a dense fibrous capsule and granulomatous inflammation is seen around deposits of silicone fluid or silicone gel. Experimental work has shown the production of contractile fibroblasts, known as myofibroblasts, around silicone implants (Rudolph et al, 1978; Rudolph & Abraham, 1980). Myofibroblasts have specific life cycles and occur during the active phase of wound healing, or, in this instance, active inflammation. Their absence in the liver and spleens examined was not unexpected as the tissues examined in the electron microscope mostly showed quiescent reactive processes.

Microembolism of silicone from solids as in cardiac prosthesis ball variance or from fluid as in breast augmentation procedures is associated with disseminated granulomata. A report of patients with silicone granulomatous hepatitis and increased levels of liver enzymes provides definite evidence that silicone-induced inflammation can produce hepatocellular damage (Ellenbogen et al, 1975). In this study 17 biopsy cases and 10 autopsy cases (3 having had previous biopsies) showed hepatic silicone and raised levels of serum AST. A total of 11 patients had chronic hepatitis. Among the cases of chronic hepatitis only one had HBsAg (Biopsy Case 7) and another two showed raised titres to cytomegalovirus (Biopsy Case 11 & 15). It is possible that the remaining 8 cases of chronic hepatitis with no recognised aetiology may be associated with non-A non-B hepatitis viruses; however, the presence of intrahepatic deposits of silicone, a proven fibrogenic and inflammatory agent, must make it a possible primary aetiologic factor. The contaminant was morphologically intimately related to the reactive process and a positive statistical correlation could be demonstrated between silicone and the severity of fibrosis and between silicone and the grade of inflammation. It is possible that silicone may also play a synergistic role in prolonging liver disease initiated by other causes. The deposition of silicone, however, was not invariably associated with raised levels of serum AST and eight out of the 22 autopsy cases showed normal levels of the enzymes.

The presence of silicone in the liver was associated with increased fibrous tissue in all instances and chronic inflammation was present in most. The reported examples of a systemic response to silicone implants with fever, arthritis and an adult respiratory distress syndrome raises the possibility of an allergic reaction. Examination of sequentially obtained liver tissues from our patients confirmed that silicone and hepatic abnormalities were not present before the onset of haemodialysis. Definitive conclusions about the progressive nature of the hepatic abnormalities cannot be made. It is an overall impression that fibrosis is progressive with accumulation of silicone; however, inflammation is probably not a progressive feature of the reaction to silicone. The initial deposition of silicone is associated with varying degrees of inflammation which tends to subside as fibrosis isolates the foreign material. It is suggested that this phasic inflammatory response may be reflected by fluctuations in the serum AST levels. The phenomenon of initial inflammation followed by progressive fibrogenesis is seen in some infective situations such as inflammatory and granulomatous mediastinitis. In this condition the fibrosis is exuberant in comparison to the inflammation and granuloma formation which frequently tend to be overlooked (Schowengerdt et al, 1969; Leong, 1978). This hypothesis is not in contradiction to animal experiments which show an initial inflammatory response that subsides as a fibrous capsule is formed around the deposit of silicone (Rees et al, 1967).

The presence of silicone particles within intravascular giant cells in the lung suggests that the circulating contaminant is phagocytosed by macrophages and stored in the mononuclear phagocytic system. At present there is no available information to indicate if silicone is at all catabolised and excreted. The large clumps and flakes of the material seen distending congested hepatic sinusoids and pulmonary vessels raised the possibility of mechanical obstruction of circulatory, renal and other vital organ systems. Careful examination of these organs, however, did not produce evidence to substantiate this suspicion. Indeed, ultrastructural examination of three renal biopsies from patients with intrahepatic deposits of silicone did not show evidence of the material in the glomeruli.

The observation of silicone particles in fat vacuoles in macrophages in some of our cases requires some comment. Fat cells around silicone cysts have been observed to be shrunken with varying degrees of atrophy. Small intracellular vacuoles of silicone have also been noted in these adipose cells. Rees et al (1967) considered the striking systemic reaction of adipose tissue to massive subcutaneous or intraperitoneal doses of silicone fluid but could not provide a satisfactory explanation.

In view of reports of foreign-body-induced sarcomas in animals (Brand et al, 1977), the question of carcinogenesis at implantation sites has frequently been raised. A recent review by Brand and Brand (1980) concludes that although a risk may exist, the actual incidence of cancer at implantation sites will remain low.

Significant amounts of silicone were deposited in the spleen, lymph node and bone marrow but depression of erythropoiesis and immune function by renal failure made it difficult to identify any functional effects that silicone may have had in these organ systems. Bommer & Ritz (1981) imply that pancytopenia may result from splenic sequestration as a consequence of splenomegaly with silicone inclusions.

X.f. SEQUEL TO THE IDENTIFICATION OF SILICONE-INDUCED CHRONIC HEPATITIS

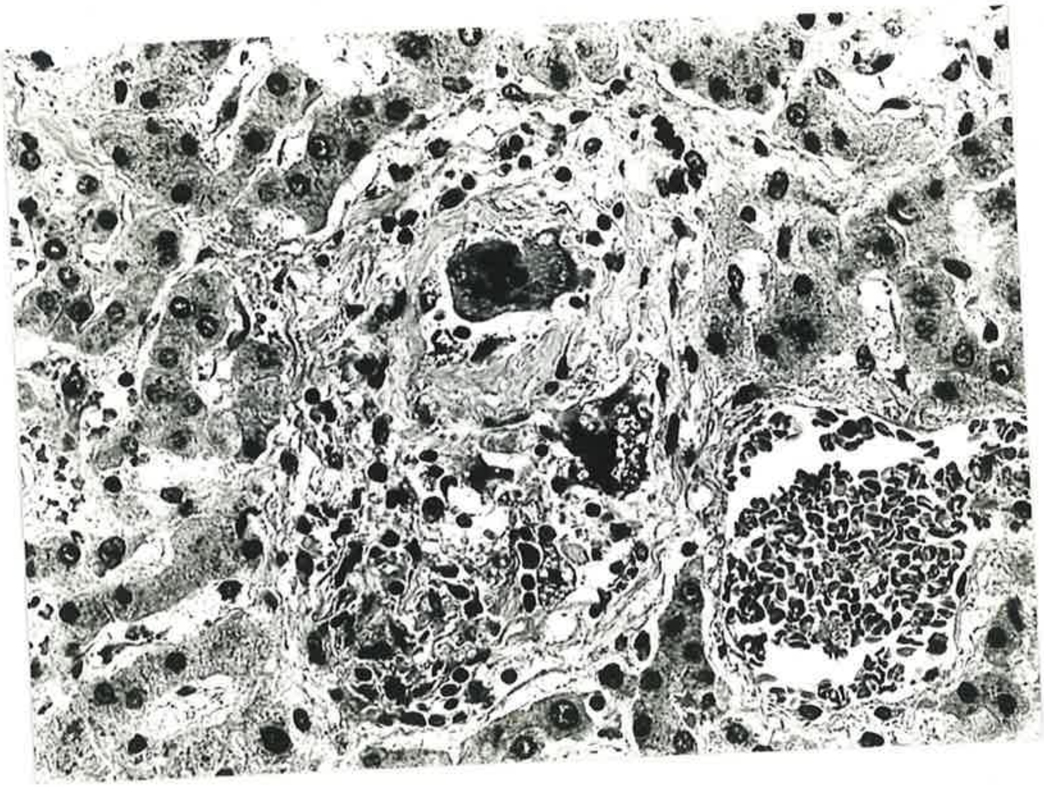
Since the substitution of the silicone tubing with PVC at the Queen Elizabeth Hospital, liver samples (1 biopsy and 4 autopsy cases) from five patients who had been dialysed for up to 12 months with only the new PVC tubings were examined. None showed silicone in the liver or other tissues.

The author has had the opportunity of seeing in consultation liver tissues from two patients dialysed for varying periods with silicone tubings at the Royal Adelaide Hospital. In both cases silicone particles were found. One of these cases came to autopsy at the Institute of Medical and Veterinary Science after a total of 14 months of haemodialysis. Silicone was found in the liver, spleen and lung sections kindly made available for examination. Atomic absorption spectrometry analysis also showed detectable levels of polydimethylsiloxane in these tissues. One patient dialysed in Victoria for 10 months and another in Canberra for 11 months came to autopsy at the Queen Elizabeth Hospital. Both cases showed silicone in the liver.

During a recent visit to Sydney Hospital the author examined the autopsy tissues from three patients who had been haemodialysed with the silicone tubing. All cases revealed the presence of silicone in their tissues (Figure 44). A fourth patient who had received only peritoneal dialysis did not show the material.

X.g. CONCLUSIONS

Mechanical stresses imposed on the silicone tubing by the roller pump can frequently produce fragmentation from its inner walls during normal use. The embolisation of this characteristic refractile, non-birefringent material produces widespread dissemination in the mononuclear phagocytic system. This newly recognised iatrogenic disorder may be a potential cause of chronic hepatic dysfunction in the renal patient. Its presence in the liver may produce a marked granulomatous hepatitis which can be associated with prolonged elevations of serum aspartic transaminase levels. The amount of



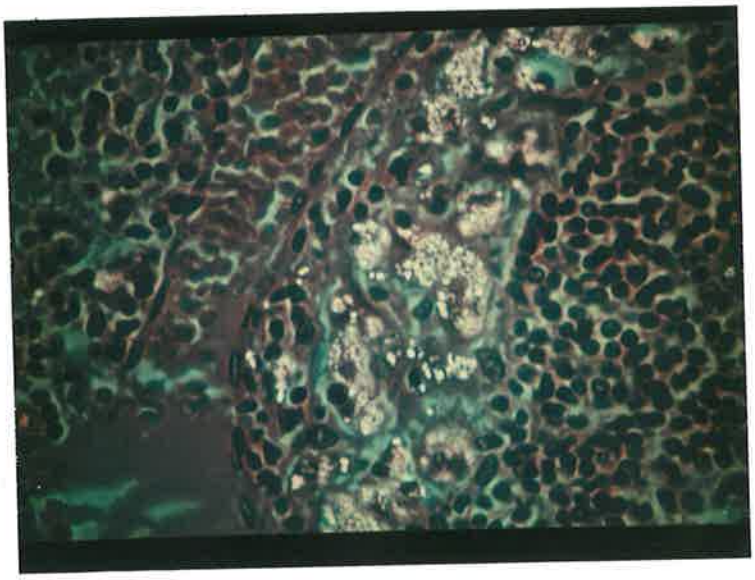


Figure 44: Photomicrograph of liver showing marked granuloma formation with refractile particles of silicone in phagocytic cells. Coloured photograph shows brightly contrast negative material in the splenic white pulp when viewed in phase contrast microscopy. 67 year old, male, dialysed at Sydney Hospital for 77 months (x 400).

silicone accumulated in the liver appears to parallel the duration of exposure and its presence is invariably associated with morphologic abnormality. Because of its non-occluding properties, silicone tubing is widely used in the roller pump segment of the haemodialysis machine. Spallation from these tubings is not the result of defective regional products as confirmed by subsequent reports from other countries and the observation of cases dialysed in other Australian hospitals. In vitro testing confirmed that spallation occurs from the silicone tubing under conditions simulating normal usage. It is strongly urged that this source of particulate contamination with its potential hazards be immediately replaced.

APPENDICES

AUSTRALIA

THE INSTITUTE OF MEDICAL AND VETERINARY SCIENCE, FROME ROAD, ADELAIDE

TISSUE PATHOLOGY DIVISION
ELECTRON MICROSCOPE UNIT

Postal Address: Box 14, Rundle Street P.O., Adelaide, S.A. 5000
Telegrams: MEDVET, Adelaide Telex: 82647 Telephone: I.M.V.S. (08) 228 7911
Electron Microscopy Enquiries: (08) 228 7522, 228 7523

Patient Name	Sex	Date of Birth	Unit Record	Ward	Date of Receipt	Laboratory Nu
I						N08486-7'
Doctor: DR A LEONG					11.4.80	TC 756

Nature of Specimen

LIVER

APPENDIX *Ia*

SUPPLEMENTARY REPORT

The specimen from Ingenito A276/79 along with an H&E stained slide was received and the tissue processed for both conventional TEM as well as for X'ray microanalysis. For X'ray microanalysis several pieces were processed without any osmium postfixation so as not to remove or alter any of the deposits seen in the H&E slide.

Light microscopy of toluidine blue sections showed segments of lobules interspersed with fibrosis and infiltration of portal areas with macrophages many of which were laden with refractile material. The granules were quite frequently seen lying in the cytoplasm of these macrophages.

At TEM level the granules were quite apparent in the macrophage cytoplasm and consisted essentially of rather amorphous looking electron opaque material. However within this amorphous matrix there were accumulation of spherical particulate materials as can be seen in Neg. 12664. Such dense particles were also seen in the intercellular areas and in the fibrosed regions. Some of the granules were also completely filled with this dense material and lacked the granular amorphous matrix. X'ray microanalysis of the unstained non osmiated material showed up the granules to consist essentially of silicon. Further detailed analysis will be made to study whether the silicon is present in combination with any other metal. But even if it is associated with any other metal it is there in very low quantity. It is however interesting to note that the background, from an area outside the cell also showed very slight traces of silicon which suggests the accumulation of silicon in the liver in rather large amounts.

COMMENT:

From experience with X'ray microanalysis of other human liver tissues and other tissues accumulation of such high amount of silicon in the macrophages seems very unusual. The nature of the silicon deposits is not like silicon dioxide which when seen in lungs or elsewhere has a needle-like configuration. It thus seems that the silicon is present in a form other than as silicon dioxide. Further studies will have to be made to find out more about the way the material has been accumulated.

Following my telephonic conversation with Dr Leong it came to light that silicon in an oily base is used extensively in the dialysis machines. The obvious inference that can be made as to the accumulation of the silicon in the liver is that it has come from the lubricant.

/ Over....(2)

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Patient Name	Sex	Date of Birth	Unit Record	Ward	Date of Receipt	Laboratory Number
I Doctor						

Nature of Specimen

CONTINUATION (2)

APPENDIX Ia

Although this cannot be stated with certainty without undertaking specific examination of the lubricant and other cases with similar liver dysfunction after long term dialysis.

The E.M. findings thus throw a new light into these accumulations in the liver and emphasizes the need for further studies.

T56000:E50940 - Liver:Silicon

JEOL :12664
X133-X139

11.4.80

TAPEN M MUKHERJEE

CC: DR A LEONG
Queen Elizabeth Hospital

PROFESSOR B VERNON-ROBERTS

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Patient Name	Sex	Date of Birth	Unit Record	Ward	Date of Receipt	Laboratory Num
K Doctor					2.7.80	N06779.80 756

Nature of Specimen

REPORT: APPENDIX Ia

For both samples with accession numbers of N06779.80 and N06780.80 with names of K and R respectively.

The sample consisted of two paraffin blocks along with appropriate H&E stained slides. The areas marked on the slides were enucleated and deparaffinised with xylol and processed with acid without osmium for x-ray microanalysis.

At light microscopy examination of toluidine blue sections showed some crystalline material apparent in the portal tract regions. These were not refractile with polarising light.

Electron microscopical examination at TEM level failed to reveal any of the microcrystalline material seen in light microscopy. Instead there were cytoplasmic dense bodies. These dense bodies as well as all other areas in the sections which could be construed to have a crystalline appearance were subjected to extensive x-ray microprobe analysis.

No specific peaks were detectable in any of the samples.

Comment:
Previous experience with I has shown that the accumulations of particulate material were electron opaque in tissue unfixed in osmium tetroxide. None of the liver showed anything remotely suggestive of the particulate material seen in I . Thus, it is not surprising that no significant peaks were observed in these two cases. It may be that the material in this instance is organic or whatever inorganic element was present had been extracted during the preparatory procedure thus precluding the possibility of an effective analysis.

Unfortunately fresh tissue is always better for analysis work, which it is admitted, is not possible in this instance. However, this possibility should be borne in mind during future samplings. I is being persued further as per request.

T56000:M09010
Tissue unsatisfactory for proper EDX assessment, liver.

31.7.80:MR T MUKHERJEE

CC: DR A LEONG
THE QUEEN ELIZABETH HOSPITAL
WOODVILLE SA 5011

THE INSTITUTE OF MEDICAL AND VETERINARY SCIENCE, FROME ROAD, ADELAIDE

AUSTRALIA

TISSUE PATHOLOGY DIVISION
ELECTRON MICROSCOPE UNIT

Postal Address: Box 14, Rundle Street P.O., Adelaide, S.A. 5000
Telegrams: MEDVET, Adelaide Telex: 82647 Telephone: I.M.V.S. (08) 228 7911
Electron Microscopy Enquiries: (08) 228 7522, 228 7523

Patient Name	Sex	Date of Birth	Unit Record	Ward	Date of Receipt	Laboratory Num
R					2.7.80	N06780.8
Doctor						756

Nature of Specimen

APPENDIX Ia

See report N06779.80.

T56000:M09010
Tissue unsatisfactory for proper EDX assessment, liver.

31.7.80:MR

T MUKHERJEE

CC: DR A LEONG
THE QUEEN ELIZABETH HOSPITAL
WOODVILLE SA 5011

UNIVERSITY OF SASKATCHEWAN

SASKATOON, CANADA
S7N OWO

February 6th 1980

Dr. A. S-Y. Leong,
Staff Pathologist,
The Queen Elizabeth Hospital,
Woodville, South Australia 5011

Dear Dr. Leong,

The electronmicrographs are not too informative. It is likely that the 'holes' in the lysosomes within the macrophages (Kupffer cells) represent the site where the material was deposited.

I therefore passed your material to Dr. Cunningham who has considerable experience in the field of kidney diseases and dialyis. I enclose herewith his report which I trust will be of value to you.

Kind regards.

Yours sincerely,

F.N. Ghadially, M.D.,Ph.D.,D.Sc.(Lond.)FRC Path., FRCP(C).
Professor and Joint-Head of Pathology,
Room A216, Health Sciences Building,
University of Saskatchewan,
Saskatoon, Saskatchewan, Canada S7N OWO

F#3003

UNIVERSITY HOSPITAL
Saskatoon, Saskatchewan.

MEMORANDUM

APPENDIX Ib

TO: Dr. F.N. Ghadially **DATE:** February 5, 1980

FROM: Dr. T.A. Cunningham **FILE NO:**

The sections sent to you by Dr. Leong are very interesting. They show a rather massive Kupffer cell and portal zone macrophage accumulation of homogenous amorphous material. In places this has proceeded to granuloma formation. I do not recall having seen this phenomenon, but we are presently engaged in reviewing some autopsies from known long-term dialysis cases.

It would have been helpful to know whether the dialysis was hemodialysis or peritoneal dialysis and whether this amorphous material was to be found in other parts of the RE system as well. If it were exclusively hepatic, there would be a strong presumption of a portal zone origin for the material. It is possible that peritoneal dialysis might result in such deposits, though one would think the lymphatics would be rather heavily involved as well. If hemodialysis has been involved, one would expect the brunt of the lesion would have been pulmonary but that there would certainly be multisystemic deposits. In either case, some phase contrast examinations of millipore filtrate of the dialysis fluid might well reveal the source of the material in sufficient quantities to identify chemically and to eliminate the source. I should be reluctant to consider an endogenous source until this had been excluded.

T.A. Cunningham, M.B., Ch.B.,
F.R.C.P. (Ed)
Department of Pathology

TAC/lm

Université Libre de Bruxelles
Laboratoire d'Anatomie Pathologique
et de Microscopie Électronique
Prof. P. DUSTIN
Rue aux Laines 97
B - 1000 Bruxelles

Brussels, June 24, 1980.

Professor J.V. Johannessen,
Department of Pathology,
The Norwegian Radium Hospital,
Oslo 3, Norway.

APPENDIX Ic

Dear Jan,

I have studied the sections you sent me from Australia. I think this refractile material is most probably thorium dioxide (thorotrast). As you know, this has been widely used in some countries (Germany, Portugal) and remains indefinitely in the tissues. There is a relatively easy method to be sure of this : radioautography, thorium emitting several strongly ionizing alpha particles.

I think this diagnosis is not in contradiction with the aspects of electron microscopy. I do not think that these cases are suitable for publication, unless the presence of thorium dioxide could not be confirmed.

Yours sincerely,

Prof. P. Dustin.

I. INSTITUTE OF PATHOLOGY AND EXPERIMENTAL CANCER RESEARCH
SEMMELWEIS MEDICAL UNIVERSITY
Budapest VIII., Üllői út 26.
Tel.: 130-669
HUNGARY
1085

I. INSTITUT FÜR PATH. ANATOMIE UND EXPERIMENTELLE KREBSFORSCHUNG DER SEMMELWEIS MEDIZINISCHEN UNIVERSITÄT
Budapest VIII., Üllői út 26.
Tel.: 138-669
UNGARN
1085

Director: Prof. Dr. KÁROLY LAPIS

Budapest, 16th July 1980

Jan Vincents Johannessen, M.D.
Department of Pathology
The Norwegian Radium Hospital
OSLO 3, NORWAY

APPENDIX Id

Dear Jan,

I have received with thanks your letter of 18th June together with the copy of Dr. A. S-Y Leong's letter sent for you. Furthermore, I have got the four sections stained by heamatoxylin eosin, as well as the six EM paper pictures.

Thank you for the honourable invitation to summarize my remarks on the material. I have studied carefully the sections and the EM pictures. In accordance with dr. Leong's description there is really in the liver, in the histiocytes of the portal tract refractile non--birefringent material to be found, sorrounded by inflammatory reaction, in some places by granulomatous response. In the post-mortem material, in the macrophages of the spleen this material can be found, as well. According to the EM pictures it seems that the material in the histiocytes, macrophages is located within extremely large secondary lysosomes merging into each other. The strange material is to be seen in the form of electronlucent areas of various shape or in the form of vacuolums which is sorrounded by granular electron dense in some places. Seeing the slides and the EM pictures and in the awareness of the anamnesis I have thought that the material in question is originated from the filters applied in haemodialysis. I have realized the base material of these filters is the so called cuprophane. Filter fragments made from cuprophane were studied by polarisation microscopy, but it proved to be birefringent, in contrast to the material found in Leong's slides. However, the possibility is arisen that the cuprophane in the organism is exposed to modification, perhaps its birefringent is lost. It seems to be unlikely. It is no doubt, for the identification of the material's characteristics the most appropriate would be the electronprobe microanalysis. If dr. Leong will supply us with the proper material we will be glad to carry out electronprobe microanalysis. I was considering to rubb off one part of one of the sections stained by haematoxylin eosin in order to be the subject to electronprobe analysis, but I have not been authorized to use any from the material for investigation. It would be also important to know if the patient was drug_addict, because as it is known, by drug-addicts talc may get into vascular system and deposited in the tissues. Otherwise, it is really stratling how severe granulomatous inflammation and reorganisation occur in the liver as the effect of this material. The disclosure of its nature is of prime sanitary importance. It is important, as well, to make this phenomenon in a wide circle of doctors known, hence I suggest it for publication in the journal Ultrastructural Pathology, even if its nature is not previously cleared.

Enclosed I return to you with thanks the material made for me available.

With the best wishes and kindest regards.

Sincerely yours,

/Dr. K. Lapis/

I. INSTITUTE OF PATHOLOGY AND EXPERIMENTAL CANCER RESEARCH
SEMMELWEIS MEDICAL UNIVERSITY
Budapest VIII., Üllői út 26.
Tel.: 138-669
HUNGARY
1085

I. INSTITUT FÜR PATH. ANATOMIE UND EXPERIMENTELLE KREBSFORSCHUNG DER SEMMELWEIS MEDIZINISCHEN UNIVERSITÄT
Budapest VIII., Üllői út 26.
Tel.: 138-669
UNGARN
1085

Director: Prof. Dr. KÁROLY LAPIS

Budapest, 5th January 1981.

Anthony S-Y Leong
MBBS, FRCPA, FCAP, MRCPATH
Senior Consultant Pathologist
The Queen Elizabeth Hospital
Woodville, South Australia 5011
Histopathology Department

APPENDIX Ie

Dear Dr. Leong,

Thank you for your kind letter of November 8, 1980. I also received your letter of August 19th together with the sent material. Unfortunately at that time our microprobe analyser was out of function, this is what caused the delay.

As can be seen from the enclosed diagnosis, we were unable to demonstrate the presence of any foreign material in the samples.

Enclosed I am sending electronmicroscopic pictures, too, which were prepared from purposely thicker sections. They show the fields where the studies were carried out with the microanalyser.

Thank you for your kind confidence and the sending of the interesting case. I am sorry we were unable to help in solving the problem. As shown by your mass chromatography studies, it is probable that the material is of chlorine nature.

Please allow me to wish you a Happy New Year,

With kindest regards,
Sincerely yours,

Dr. K. Lapis

Enclosure

Materials and Methods: APPENDIX Ie

Liver specimen no.: 16069: embeding in Durcupan

fixation : 2% Glutaraldehyde

+4% formaline

2% OsO_4

contrast: U + Pb

Thickness of section 1500 - 2000 Å / 50 Å /vapoured with carbon/

Apparatus: JEOL JEM-100 C scanning transmission electronmicroscope combined with ORTEC 6230-type microanalyzator /EDS system/

Copies of photographs: No. 147932 - 147942 /11 copies/

Copies of X-rays: No. 72 - 77 /6 copies/

The spectrums analyseed from the sections only contain the Os; U and Pb spectrums of the fixative and contrasting materials - besides the supporting grid.

The presumed Cl should have been demonstrable at the light/pale line, where it could not be demonstrated significantly.

Budapest, 1980. dec. 22.

The Australian
neral Development
Laboratories

ıgton Street, Frewville.
South Australia 5063
ıone Adelaide 79 1662
Telex AA 82520

Please address all
correspondence to
.O. Box 114 Eastwood
SA 5063
In reply quote:

amdel

24 April 1981

APPENDIX II*a*

GS 4/0/0

Queen Elizabeth Hospital,
Histo-Pathology Department,
Woodville Road,
WOODVILLE, SA 5011.

Attention: Dr A. Leong

REPORT GS 4864/81

YOUR REFERENCE:	AMDEL Service Work Application form dated 3 April 1981
MATERIAL:	Three blocks with embedded liver tissue
IDENTIFICATION:	F33061e rat, 27580g J ; and 19879b I
DATE RECEIVED:	3 April 1981
WORK REQUIRED:	Determination of silicon contents

Investigation and Report by: Peter Schultz

Manager, Geological Services Division: Dr Keith J. Henley

for Norton Jackson
Managing Director

jd/1

Plant: Osman Place
Thebarton S.A.
Telephone 43 8053
ıranch Laboratories:
Perth W.A.
Telephone 325 7311
Melbourne Vic.
Telephone 645 3093

APPENDIX IIa

ELECTRON MICROPROBE ANALYSIS OF LIVER TISSUE

1. INTRODUCTION

Three blocks with embedded liver tissue, marked F33061e rat, 27580g Jeffries and 19879b Ingenito, were received for electron microprobe analysis of the liver tissue for the presence of silicon.

The rat liver was known to contain silicon, while the two human livers were suspected to contain silicon in submicron-sized particles.

2. EXPERIMENTAL PROCEDURE

The three blocks containing the livers were cut down to a height of under 7 mm and carbon coated to make them electrically conductive. The livers were then analysed using a low intensity beam to minimise sample damage.

3. RESULTS

The rat's liver showed high concentrations of silicon in a number of areas, while the human livers showed some silicon concentration in several tiny spots. The particles containing the silicon were too small to be resolved with the electron probe.

The Australian
[Mi]neral Development
Laboratories

[Osm]ington Street, Frewville,
South Australia 5063
[Ph]one Adelaide 79 1662
Telex AA 82520

Please address all
correspondence to
[P.]O. Box 114 Eastwood
SA 5063
In reply quote:

amdel

APPENDIX IIb

4 June 1981

GS 4/0/0

Queen Elizabeth Hospital,
Electron Microscope Unit,
Woodville Road,
WOODVILLE, SA 5011.

Attention: Dr David Gove

REPORT GS 4864/81 - ADDENDUM

YOUR REFERENCE:	AMDEL Service Work Application form dated 3 April 1981
MATERIAL:	Three blocks with embedded liver tissue
IDENTIFICATION:	F33061e rat, 27580g J[illegible] and 19879b I
DATE RECEIVED:	3 April 1981
WORK REQUIRED:	Determination of silicon contents

Investigation and Report by: Peter Schultz

Manager, Geological Services Division: Dr Keith J. Henley

for Norton Jackson
Managing Director

jd/1

Plant: Osman Place
Thebarton S.A.,
Telephone 43 8053
[B]ranch Laboratories:
Perth W.A.
Telephone 325 7311
Melbourne Vic.
Telephone 645 3093

ELECTRON MICROPROBE ANALYSIS OF LIVER TISSUE

1. INTRODUCTION

The three liver tissues, marked F33061e rat, 27580g Jeffries and 19879b Ingenito, which were analysed previously and reported in AMDEL report GS 4864/81, dated 24 April 1981, were re-examined with a view of obtaining photographs and charts of the silicon distribution in the livers.

2. EXPERIMENTAL PROCEDURE

An electron probe of about 1 to 2 micron diameter, generated by an accelerating voltage of 15 kV and giving a specimen current of about 15 nA was used to obtain absorbed electron and SiKα images of areas containing silicon. The magnification used was ×1200 giving images of about 120 × 120 μm. The exposure times used for the SiKα images was one minute for the rat liver, and fifteen minutes each for Jeffries and Ingenito livers.

Spectrometer scans were also carried out across the SiKα peaks in the three livers with the beam being stationary on high silicon spots.

3. RESULTS

Scanning the liver tissues with the electron beam results in some surface damage so that no too much weight should be given to the interpretation of the scanning electron images. The SiKα images show genuine high silicon spots, but the general low level of spots is due to some background radiation generated with the characteristic SiKα radiation. The negatives obtained are being sent by separate mail together with the samples. The explanation of the negatives is as follows:

12A - rat (absorbed electron image)

13A - rat (SiKα)

14A - Ingenito (absorbed electron image)

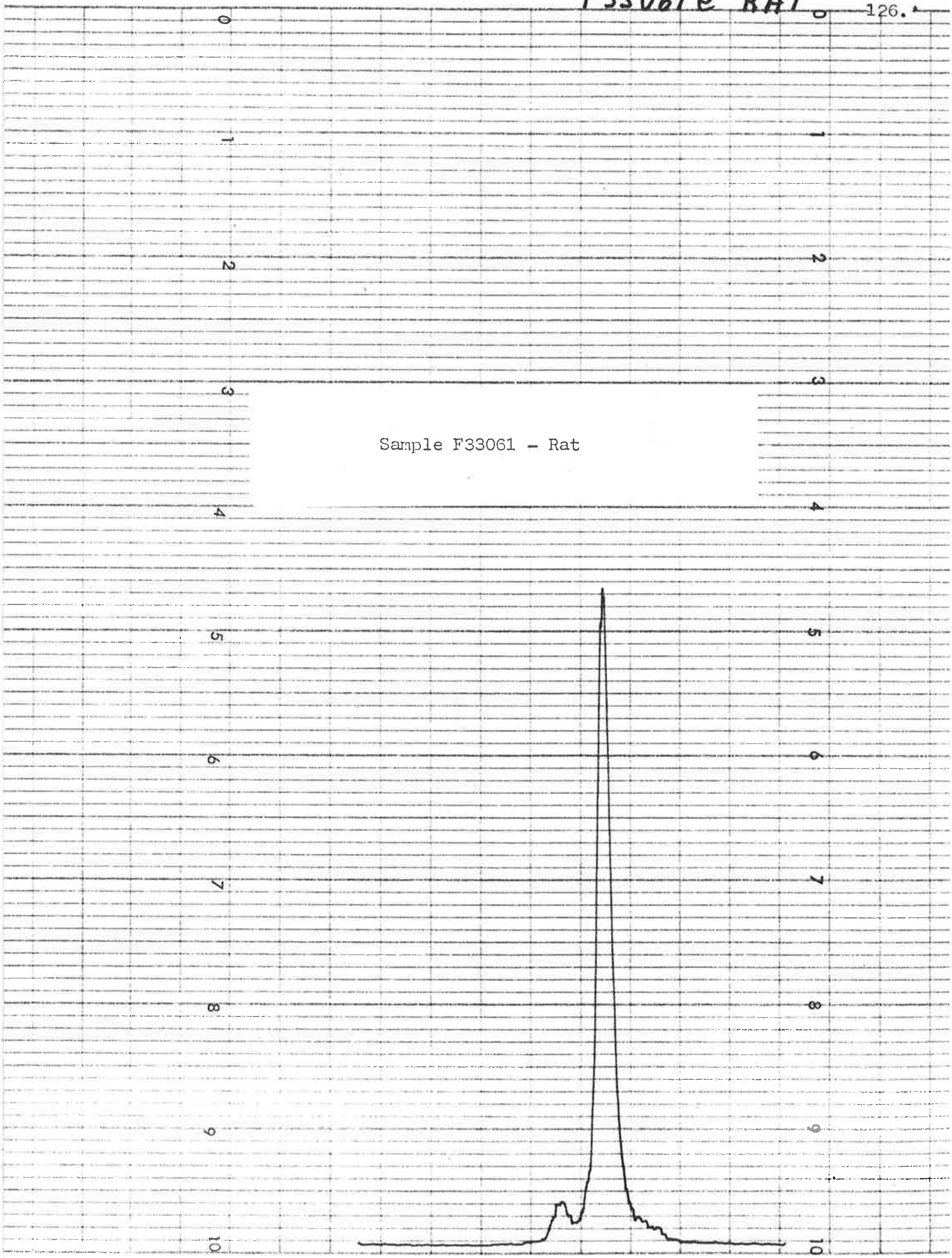
15A - Ingenito (SiKα)

16A - Jeffries (absorbed electron image)

17A - Jeffries (SiKα)

The spectrometer scans through the SiKα peak were carried out at $^1/_8$ two theta degrees per minute, with a chart speed of ½ cm per minute and a counting rate (C.R.) of 3×10^3 counts per minute and the accompanying time constant (T.C.) of 10 seconds for the Ingenito and Jeffries livers while for the rat liver the spectrometer rate was ½ °/minute with a chart speed of 2 cm/minute, a C.R. of 3×10^4 c.p.m. and T.C. = 2 seconds. It is therefore seen that the silicon concentration in the rat liver is about ten times higher than in the two human livers. The three charts are enclosed with the results.

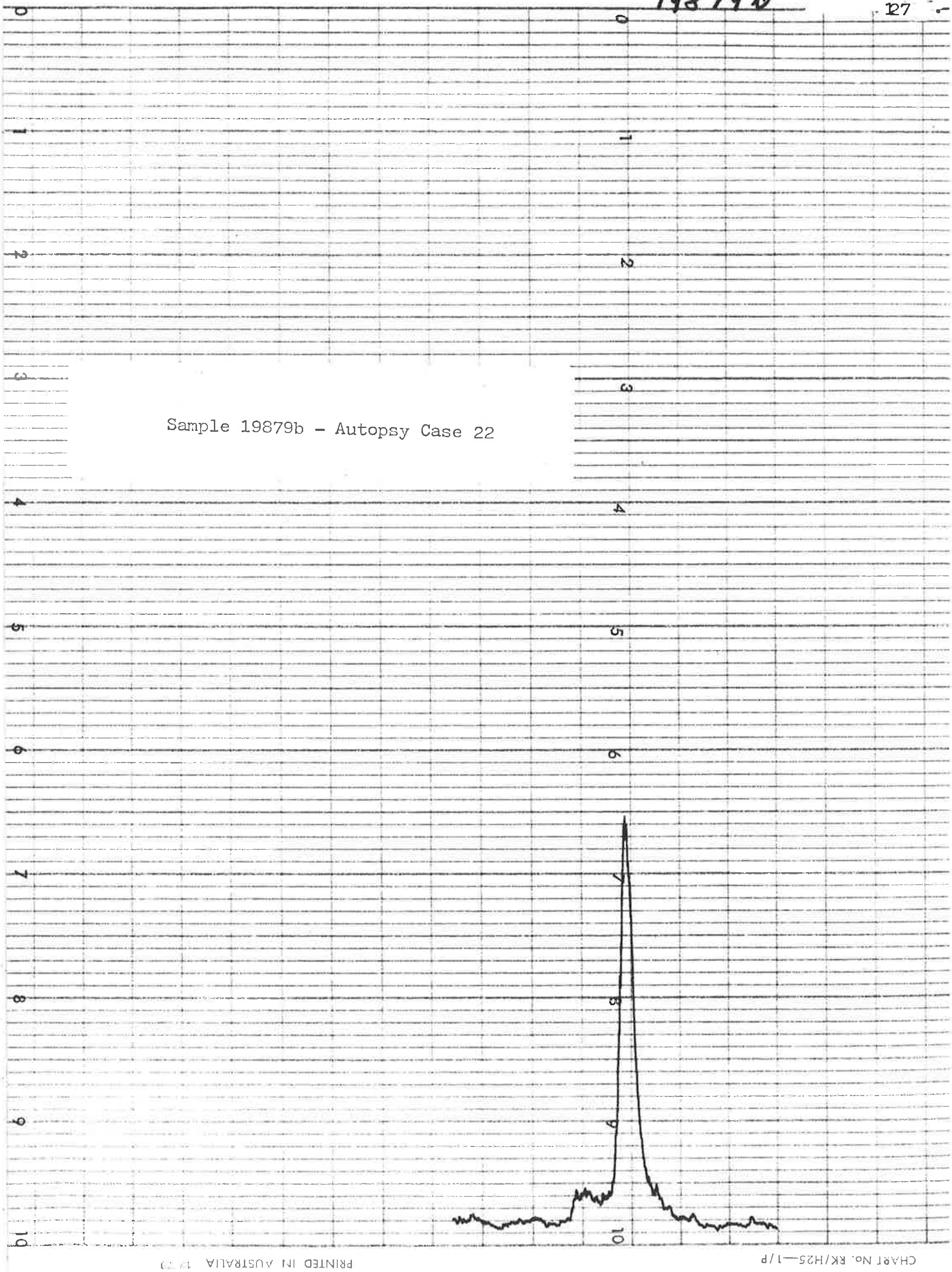
F33061e RAT



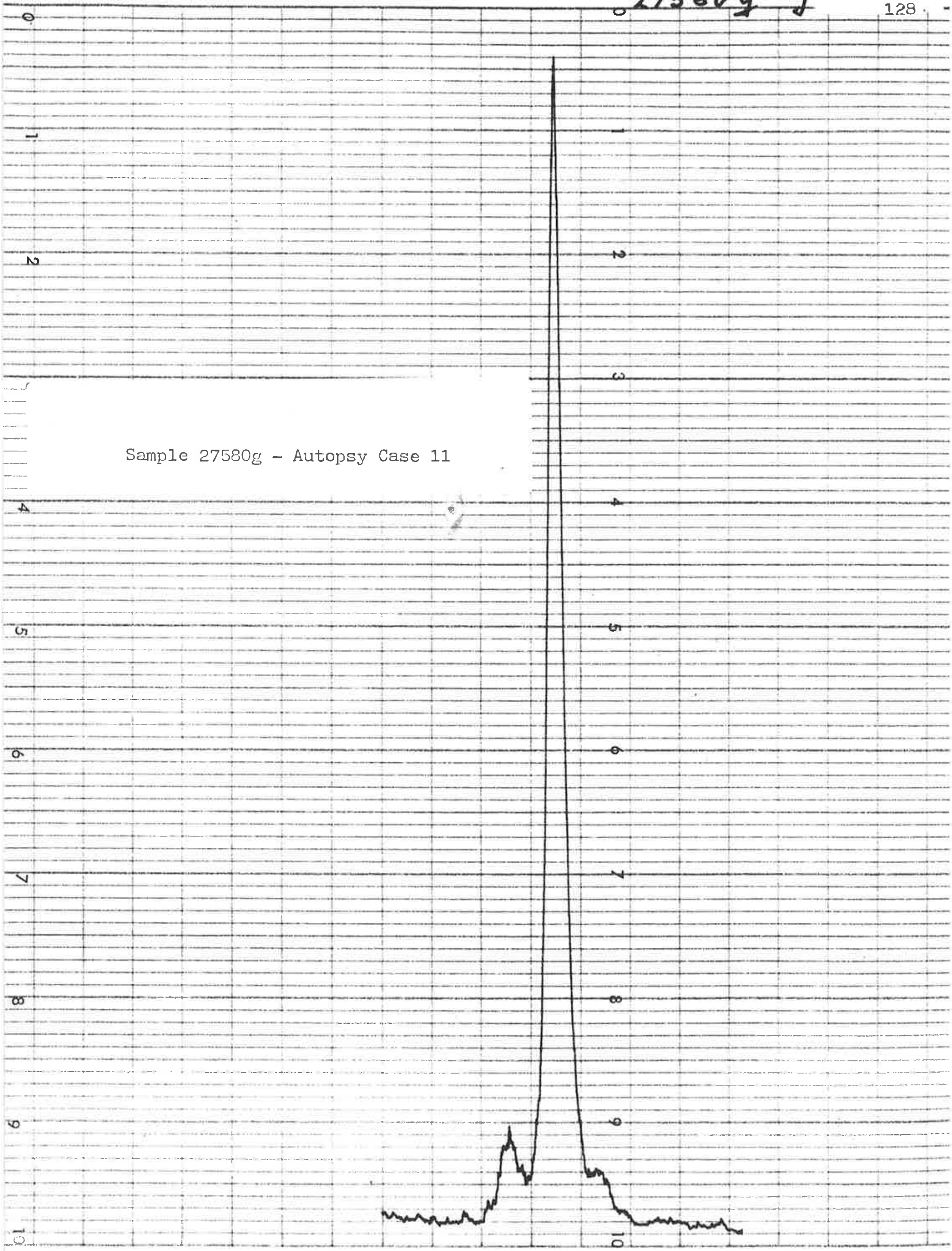
Sample F33061 - Rat

CHART No. RK/H25—1/P

19879b



Sample 19879b - Autopsy Case 22



Sample 27580g - Autopsy Case 11

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SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY, M-012

LA JOLLA, CALIFORNIA 92093

April 30, 1981

Anthony S.Y. Leong, M.D.
Histopathology Department
Queen Elizabeth Hospital
Woodville, Adelaide
South Australia 5011

Dear Doctor Leong:

I read with interest the letter in Lancet (April 18) regarding particles in liver of dialysis patients. I have had some experience with silicone, etc., in tissues and would be glad to analyze the particles if you feel it is worthwhile. Silicone would show a peak for silicon with microprobe analysis. I have not observed similar material here except in intravenous drug users (who may be analogous to the dialysis patients).

Looking forward to your reply. I would need only a paraffin block for the analysis.

Sincerely,

Jerrold L. Abraham, M.D.
Assistant Professor
Department of Pathology

JLA:bl

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SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY, M-012

LA JOLLA, CALIFORNIA 92093

June 29, 1981

Dr. Anthony S-Y Leong
Senior Staff Pathologist
Queen Elizabeth Hospital
Woodville, S. Australia 5011

Re: Your letter of 5/11 and my sample # JA 81-60, 61, 62 and 63

Dear Dr. Leong:

Thank you so much for sending the tissue samples as well as the segment of silastic tubing. I have been able to analyze two of the samples so far and it has been easy to demonstrate silicone in both samples (J.   and I   ). I am enclosing a few scanning electron micrographs from the latter case, in which it was much easier to find the material using backscattered electron imaging. It is of interest that in both cases, in addition to the silicone, there was abundant evidence of endogenous calcification, with calcium and phosphorous being detectable in many of the inclusions which have a blue appearance by hematoxylin and eosin stains. I assume both of these patients were in poor balance as far as calcium and phosphorous were concerned.

I think this would be worth writing a followup report to The Lancet and with your permission I would prepare a letter containing these figures, plus the other portion which I hope to have prepared by the next few weeks, namely, the portion of the tubing, to see if similar sized small particles of silicone are evident on its surface. If the small particles are not on the surface, they may be formed by abrasion as the tubing is connected with other sets of tubing.

I look forward to hearing from you. Best regards.

Sincerely,

Jerrold L. Abraham, M.D.
Assistant Professor
Department of Pathology

JLA:gr

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SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY, M-012

LA JOLLA, CALIFORNIA 92093

September 3, 1981

Anthony S.-Y. Leong, M.D.
Queen Elizabeth Hospital
Woodville, S. Australia 5011

Dear Dr. Leong:

Thank you for your letter of August 25th. I am sorry the original material had not reached you yet. In case it was sent by surface mail or lost, I am enclosing a few more prints of what I had sent you. Had it reached you as planned, I had hoped you might be able to include it in your report. I had hoped to assist you with your report. I do not think it needs to be reported separately if you have similar data already. What I may do is request your permission to use it in some future series of cases dealing with microanalysis and/or silicone in tissues. Please let me know your thoughts about an additional report dealing with further analytical information or whatever. If your analytical laboratory does not have the capability of baackscattered electron imaging it may be that the micrographs I have sent will illustrate the materials in the sections more clearly and only the secondary electron image.

With best regards, I am

Sincerely,

Jerrold L. Abraham, M.D.
Assistant Professor
Department of Pathology

JLA:gr
encl.

University of Pittsburgh

SCHOOL OF MEDICINE
Department of Medicine
Division of Cardiology

April 16, 1981

Dr. A. S. Y. Leong
MBBS, MRC
Path, FRCPA, FCAP
Department of Histopathology
The Queen Elizabeth Hospital
Woodville, SA. Australia, 5001

Dear Dr. Leong:

Please excuse the lateness of this reply regarding the analysis of silicone. Listed below is the full address of Skinner and Sherman, Inc. who performed our analyses of silicone. I contacted Mr. Ralph Berger, the President of Skinner and Sherman on your behalf to ask him about the actual methods utilized in analysis. He stated that some of them are actually proprietary and probably can not be shared with you. However, I told him that you would be writing regarding your interest in the subject and he anticipated a letter from you. I should add that, as you know from our article, we analyzed for the presence of silicone in sputum, urine, blood, and breast fluid. The biochemist at Skinner and Sherman assured me that these analyses were extremely accurate and was quite sure that the interpretation was correct. In my attempt to get to the bottom of my patient's problem, I found this laboratory to be extremely helpful and I hope that your experience is similar. I should add that the analyses we undertook were quite extensive, ranging in the area of $300-700, depending on the particular study.

I hope that this information has been of some help to you.

Most sincerely,

Barry F. Uretsky, M. D.

BFU/efe

Skinner and Sherman Laboratories
300 Second Avenue
P. O. Box 521
Waltham, MASS 02254 (Telephone: 617-890-7200)

CARDIAC DIAGNOSTIC LABORATORIES, 3411 PRESBYTERIAN UNIVERSITY HOSPITAL
PITTSBURGH, PA 15213 (412) 647-3430

The Australian
[...]neral Development
Laboratories

[...]ngton Street, Frewville,
South Australia 5063
[...]hone Adelaide 79 1662
Telex AA 82520

Please address all
correspondence to
[...]O. Box 114 Eastwood
SA 5063
In reply quote:

amdel

4/0/0 - AC 4580/81

22 April 1981

NATA CERTIFICATE

APPENDIX IIe

Dr. Anthony Leong,
The Queen Elizabeth Hospital,
WOODVILLE S.A. 5020

REPORT AC 4580/81

YOUR REFERENCE: Request dated 20 March 1981

IDENTIFICATION: As listed

DATE RECEIVED: 20 March 1981

D.K. Rowley
Manager
Analytical Chemistry Division

for Norton Jackson
Managing Director

ij

[...]t Plant: Osman Place
Thebarton S.A.
Telephone 43 8053
[...]nch Laboratory: Perth

Report AC 4580/81
Page 2

ANALYSIS

µg/g
(AS RECEIVED) SILICONE

SAMPLE MARK	SILICONE*
Control	<10
J (name deleted - A11)	20
I (name deleted - A22)	150

* NOTE: The results are chloroform soluble silicon expressed as dimethyl polysiloxane.

The Australian
neral Development
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ngton Street, Frewville,
South Australia 5063
hone Adelaide 79 1662
Telex AA 82520

Please address all
correspondence to
.O. Box 114 Eastwood
SA 5063
In reply quote:

amdel

NATA CERTIFICATE

4/0/0 - AC 5200/81

16 September 1981

APPENDIX IIf

Dr. T. Leong,
Queen Elizabeth Hospital
WOODVILLE S.A. 5011

REPORT AC 5200/81

YOUR REFERENCE:	Request dated 27 April 1981
IDENTIFICATION:	As listed
DATE RECEIVED:	27 April 1981

D.K. Rowley
Manager
Analytical Chemistry Division

for Norton Jackson
Managing Director

ij

t Plant: Osman Place
Thebarton S.A.,
Telephone 43 8053
Branch Laboratories:
Perth W.A.
Telephone 325 7311
Melbourne Vic.
Telephone 645 3093

MASS SPECTROMETRY RESULTS ON SELECTED SAMPLES

The blood samples were unsuitable for mass spectrometry. Both the silastic tubing and the liver sample marked "ingenito" were suitable. However no mass spectrum of silicone polymer could be obtained due to its high molecular weight. Consequently the mass spectrums neither prove or disprove that the silicone found in the liver is the same polymer as that found in the silastic tubing.

A small amount of plasticizer was found in the silastic tubing but not in the liver, this is most likely due to contamination (from polythene packing) because plasticizer is not generally found in silastic tube.

The mass spectrums were obtained from the same chloroform extract used for quantitative silicone analysis.

Report AC 5200/81
Page 3

ANALYSIS

µg/g (as received) silicone

SAMPLE MARK	ORGAN	SILICONE µg/g	SILICONE µg/1
NAME			
T (A16)	Liver	15	
T.	Spleen	70	
T.	Lung	15	
T	Lymph Node Fat	15	
T	Lumph Node Meat	50	
K (A21)	Liver	45	
M. (C1)	Liver	<10	
S. (A20)	Liver	30	
S.	Spleen	80	
B (C2)	Liver	15	
N. (C3)	Liver	<10	
B (A1)	Liver	20	
P. (A10)	Liver	<10	
F (A4)	Liver	15	
I (A22)	Liver	160	
Blood	20 hrs		350
Blood	48 hrs		800
Blood	96 hrs		1100

ALL NAMES HAVE BEEN DELETED AND SUBSTITUTED WITH CODES

A = Autopsy, C = Controls

APPENDIX III

(1)

KENDALL COEFFICIENT TEST

Si vs. Inflammation

Si	Inflammation
2.00	0.00
3.00	2.00
1.00	2.00
1.00	2.00
2.00	2.00
3.00	1.00
2.00	1.00
2.00	2.00
3.00	3.00
2.00	1.00
4.00	3.[illegible]
[illegible].00	1.[illegible]
[illegible].00	2.[illegible]
[illegible].00	[illegible]
[illegible].00	[illegible]
[illegible].00	2.[illegible]
4.00	2.[illegible]
3.00	3.[illegible]
2.00	1.[illegible]
1.00	0.00
2.00	1.00
3.00	3.00
1.00	0.00
3.00	1.00
1.00	2.00
4.00	2.00
3.00	2.00
2.00	3.00
1.00	1.00
3.00	3.00
3.00	3.00
1.00	0.00
3.00	[illegible].00

N= 33
K= 198
K*= 3.17791086364

$p = 0.002$

Kendall Coefficient Test: SILICONE vs INFLAMMATION

APPENDIX III

(2)

KENDALL COEFFICIENT TEST

S	vs. F.b
2.00	1.00
3.00	3.00
1.00	2.00
1.00	2.00
2.00	2.00
3.00	2.00
2.00	2.00
2.00	1.00
3.00	2.00
2.00	3.00
4.00	3.00
2.00	1.00
3.00	3.00
4.00	2.00
3.00	2.00
3.00	2.00
4.00	4.00
3.00	3.00
2.00	3.00
1.00	1.00
2.00	1.00
2.00	2.00
3.00	2.00
1.00	1.00
3.00	2.00
1.00	2.00
4.00	2.00
3.00	2.00
1.00	2.00
3.00	1.00
3.00	4.00
1.00	1.00
3.00	2.00

N= 33
K= 150
K*= 2.44853150054

$p = 0.02$.

SILICONE VS FIBROSIS

APPENDIX III

Silicone vs Duration of Haemodial.

(3)

v KENDALL COEFFICIENT TEST

Duration vs. Si.

Duration	Si
9.00	4.00
28.00	3.00
69.00	3.00
84.00	4.00
27.00	3.00
18.00	2.00
13.00	1.00
10.00	1.00
51.00	2.00
28.00	3.00
9.00	1.00
31.00	3.00
6.00	1.00
42.00	4.00
7.00	3.00
38.00	2.00
7.00	1.00
43.00	3.00
54.00	3.00
10.00	1.00
36.00	3.00
20.00	2.00
39.00	3.00
54.00	3.00
8.00	1.00
6.00	1.00
6.00	2.00
22.00	3.00
30.00	3.00
3.00	0.00
17.00	2.00
3.00	1.00
18.00	2.00
28.00	3.00
10.00	1.00
17.00	2.00
52.00	4.00
15.00	2.00
15.00	3.00

N= 39
K= 383
K*= 4.86313545909

$p < 0.0001$

B I B L I O G R A P H Y

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