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*Abstract.* In a four-year period three isolated cases of hepatitis were observed within a period of 2 months in a haemodialysis unit. The cases occurred in 3 patients during testing of new haemodialysis equipment. Liver biopsy in 2 patients showed non-specific hepatitis and changes as in "viral hepatitis", respectively. From the tested blood tubings, manufactured from polyvinylchloride (P.V.C.), diaethylphthalate was washed out in an amount of 10–20 mg per litre aqueous perfusate. From the tubings normally used in the department this compound was not released on perfusion, and the symptoms disappeared rapidly when treatment with the use of these tubings was resumed.

Endemic outbreaks of hepatitis in haemodialysis units have repeatedly been reported (Annotation, Lancet, 1965; Nyström & Ringertz, 1967; London, DiFiglia, Sutnick & Blumberg, 1969). Some of these series undoubtedly represent examples of inoculation hepatitis. However, during the dialysis treatment the blood of the patients is intermittently exposed to contact with foreign materials: membranes, tubings, connections etc. These materials may not be as inert as supposed by Gesler & Kartinos (1970) and a discussion of possible toxic effects of substances exuding from P.V.C. has been published (Duke & Vane, 1968; Cowper, 1968; Little, 1968; Jaeger & Rubin, 1970).

During the months September 1969 to February 1970 we have made some clinical observations following the use of a new set of P.V.C. blood tubings in haemodialysis. As a preliminary conclusion from our observations and from some experimental investigations we would like to emphasize that a potential hepatotoxic agent may exude from P.V.C. tubings during perfusion and that jaundice in haemodialysis patients may be due to a toxic effect upon the liver.

Requests for reprints: Bent Nielsen, Section of Nephrology, Third Medical Department, Copenhagen Kommunehospital, 1399 Copenhagen K, Denmark.

## CLINICAL OBSERVATIONS

A total of 4 patients with abnormal liver function tests has been found among 27 patients with terminal renal failure, treated with regular haemodialysis in Copenhagen Kommunehospital 1966–70.

### *Case 1*

Patient J. J. W. K. was a man of 25 years with a chronic glomerulonephritis who had been submitted to intermittent haemodialysis treatment since February 1967. At the beginning of January 1969, 3 months after a bilateral nephrectomy complicated by severe bleeding, the patient developed fever, nausea and pronounced jaundice. He was isolated and the dialyses were performed in the room with the use of disposable dialysers (Alwall-Gambro type). The symptoms subsided slowly during the following 2 months. Neither liver biopsy nor tests for Au-antigen were performed.

### *Comment to Case 1*

In view of the foregoing complicated operation requiring multiple blood transfusions, a possible incubation time of about 3 months, and the course of the disease, this patient was regarded as a case of inoculation hepatitis.

No "secondary" cases were found either among the patients or among the staff despite regular control of both groups including weekly measurements of the serum concentrations of glutamic oxalo-acetic acid transaminases (GOT) and lactic dehydrogenases (LDH).

The other 3 cases differed from the first one. A planned remodelling of the unit initiated clinical trials with different "machines" for haemodialysis control. Among the equipments submitted to examination was a monitoring system (Manufacturer: Danish Sugar Company Ltd.) to which belonged a set of blood tubings manufactured from P.V.C. (Polymers produced by Montecatini, Italy).

The monitor and the blood tubings were used for 93 dialyses during 5 months (late September 1969 to early February 1970). Seventy-five of the test dialyses were carried out in only 3 patients who received a total of 18, 24 and 33 treatments, respectively, with the apparatus. These 3 patients constitute the remaining 3 cases of patients with abnormal liver function tests.

### *Case 2*

Patient O. H. was a man of 40 years with recurring nephrolithiasis and chronic pyelonephritis. The patient had been regularly dialysed since June 1967, first with peritoneal dialysis and, from August 1968, with haemodialysis. The patient felt well until December 1969 during which month he exhibited increasing nausea and dyspnoea. Hepatomegalia, slight oedemas and, on a single occasion, an increase of the serum LDH were found.

During the preceding 2 months the patient had received 14 dialyses with the use of the equipment in question. Regular control of the serum enzymes had shown normal concentrations.

The patient was considered "underdialysed" and dialysis treatment was intensified. Simultaneously a change to the unit's ordinarily used equipment accidentally occurred (Monitor: Heppell Co.; dialyser and tubings: Alwall-Gambro). The symptoms disappeared in the course of 3 weeks following this change. No liver biopsy was taken. Serum tests for Au-antigen were negative, as was the test for Au-antibodies, performed September 1970.

### *Case 3*

Patient S. B. was a girl of 25 years with a congenital renal dysplasia, maintained on regular haemodialysis treatment since June 1969. In November 1969, after 9 dialyses with the aforementioned equipment, the patient developed slight nausea and malaise, but no jaundice. An increase of the hitherto normal serum concentrations of GOT and LDH was noticed. A *liver biopsy* (December 1969) showed the lobular architecture to be preserved. In the portal tracts slight infiltration of lymphocytes and histiocytes and in the parenchyma a few scattered, fine, focal, lytic necroses with slight Kupffer cell proliferation were seen. The changes are in accordance with so-called non-specific reactive hepatitis (Per Christoffersen, personal communication). The patient was temporarily isolated and dialysis was performed with the normally employed equipment. The symptoms disappeared subsequently.

When treatment with the new monitoring system was resumed a month later the patient spontaneously remarked that low grade fever, muscle pains, nausea, and uncharacteristic abdominal pains were the regular consequences of the treatment.

After another 14 dialyses with the monitoring system the use of this equipment had to be stopped because of increasing severity of the symptoms described. At first the complaints appeared late during the dialysis and disappeared promptly following disconnection from the dialyser. With repeated dialyses both the intensity and the duration of the symptoms increased and at the time of cancelling of treatment with the apparatus the patient had severe constant pains in the back and in the upper right quadrant of the abdomen with exacerbations during dialysis. At that time she had nausea, and hepatomegalia, increased concentration of serum GOT, a serum bilirubin concentration of 2.4 mg per 100 ml and fever about 38°C were found. X-ray examination of the oesophagus, stomach and duodenum was normal. A *re-biopsy of the liver* showed the changes to be similar to those described in the biopsy 2 months earlier except that the Kupffer cell proliferation was much more pronounced (Hemming Poulsen, personal communication).

The treatment was continued with the ordinarily used equipment and the patient was well with normal laboratory values 3 weeks later. Serum tests for Au-antigen were negative, as was the test for Au-antibodies, performed September 1970.

### *Case 4*

Patient J. F. was a man of 25 years with a chronic glomerulonephritis regularly dialysed since September 1969. After having been submitted to a total of 33 dialyses with the use of the new equipment this treatment had to be cancelled because of a symptomatology that was identical with that of Case 3. The patient had a normal serum bilirubin concentration, though. A *liver biopsy* (February 1970) showed the lobular architecture to be preserved. In the parenchyma slight changes as in "viral hepatitis" with mainly centrilobular fine, focal, lytic liver cell necroses, acidophilic bodies and ballooning. No cholestasis (Per Christoffersen, personal communication). After a change to the unit's normally used equipment the symptoms disappeared during the following 3 to 4 weeks and have not recurred. Serum tests for Au-antigen were negative, as was the test for Au-antibodies, performed September 1970.

### *Comment to Cases 2, 3 and 4*

Following 10–15 dialyses with the use of a new monitoring system and blood tubings all patients complained of abdominal pains and nausea late during the dialysis. The symptoms increased in severity with increasing number of dialyses, and on cancelling of the treatment with the test dialysis system severe pains, abnormal serum levels of liver enzymes and in one case jaundice were present.

Liver biopsy in Case 3 (S. B.) showed on two occasions non-specific hepatitis with the more pronounced changes in the last biopsy. In Case 4 (J. F.) the liver biopsy revealed a histological picture compatible with a diagnosis of viral hepatitis. However, these changes were indistinguishable from those associated with certain types of toxic effect upon the liver. This patient had had the highest number of dialyses with the new equipment.

The symptoms disappeared shortly after a change to the ordinarily used monitor and blood tubings. None of the 3 patients were Au-antigen positive, and tests for the presence of Au-antibodies, performed half a year later, were likewise negative in all patients. No "secondary" cases were observed either among the patients continuously dialysed with the ordinary equipment or among the staff.

Provocation experiments were not carried out but the course of events in Case 3 showed the result of an intended reexposure of the patient to the use of the dialysis system.

### *Toxicological Considerations*

No evidence of metal corrosion or any other changes that might be held responsible for the observed clinical effects could be found on examination of the monitor. The attention therefore turned to the blood tubings made of P.V.C.

In order to make the brittle P.V.C. suitable for the purpose it has to be mixed with and to interact with a number of stabilizers, plasticizers etc. The quantity of the additives often amounts to as much as 40 to 50% of the final product. Among these compounds different phthalic acid esters, organic tin compounds, epoxidised soya bean oil, esters of dibasic $C_4$–$C_8$ acids and different organic phosphate and sebacate compounds may be listed (Brydson, 1966).

The blood tubings used showed varying degrees of discoloration of the P.V.C. ranging from a clear bluish-white colour to various brown nuances. To find out if the discoloration observed could be a result of different radiation doses, the P.V.C. tubings were exposed to a wide range of radiation doses (0 to 8 Mrad).

Furthermore, the tubings were perfused with aqueous salt solutions with salt concentrations comparable to those of serum. The aim was to find out if potentially toxic substances could be washed out of the P.V.C. during perfusion and whether the presence of such compounds could possibly be a consequence of radiation-induced disintegration of one or more of the P.V.C. components.

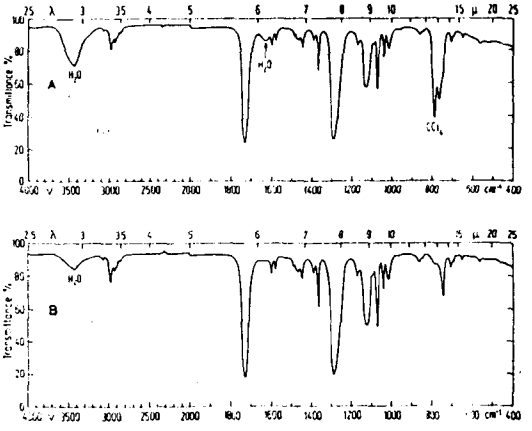


*Fig. 1.* IR-spectra of KBr discs containing the residue from evaporation of the carbon tetrachloride extracts mentioned in the text. Spectrum A is obtained from a perfusate extract, and spectrum B is obtained from the extract of a solution containing 18.6 mg diaethylphthalate per litre.

### *Experimental Procedure and Preliminary Results*

Experimental exposure of P.V.C. tubings to varying radiation doses showed the brown colour to increase in rough proportion to the increase of the irradiation dose.

Perfusion experiments were performed with one litre of sterile aqueous solution with the following composition: $Na^+$ 150, $K^+$ 4.0, $Ca^{++}$ 4.0, $Mg^{++}$ 2.0 and $Cl^-$ 160 meq/l with a pH of 6.5–7.1. Experimental conditions were varied as far as perfusion time, temperature and the irradiation dose to which the tubings had been exposed were concerned. After perfusion the fluid was quantitatively as well as qualitatively analysed with the use of both IR- and UV-spectrophotometry.

A typical example of an IR-spectrum of the substance obtained by extraction of the perfusate with carbon tetrachloride is shown in Fig. 1, spectrum A. This spectrum is not identical with the spectrum of di(2-aethylhexyl)-phthalate, the compound generally used as plasticizer in P.V.C. It proved to be identical with that of diaethylphthalate (Hummel, 1958). In order to estimate the amount of diaethylphthalate in the perfusate standard solutions of 37.2, 18.6 and 9.3 mg ester per litre salt solution were prepared and analysed. Fig. 1, spectrum B, shows the spectrum obtained from the solution containing 18.6 mg/l. The identity with spectrum A of Fig. 1 is evident.

The amounts of diaethylphathalate found by IR-analyses in the perfusates ranged from 10 to 20 mg/l. UV-determinations, using the perfusate directly, gave somewhat higher values, ranging from 20 to 50 mg/l. Other contaminants showing absorption around 280–284 $\mu$m may be included in this determination.

Neither the UV- nor the IR-analyses revealed any significant deviations in the amount of contamination which could be correlated to the radiation dose. Only a slight decrease in the amount of diaethylphthalate in the perfusate was registered after repeated perfusions. Experiments with shorter perfusion times—1 hour and 3 hours—indicated that practically all the contamination takes place in the beginning of the perfusion.

The most surprising result of the IR spectrophotometric analyses of the perfusate is the fact that the only contaminant found by us was diaethylphthalate which is not the main constituent of the plasticizer. An attempt has therefore been made to identify the plasticizer used in the P.V.C. tubings in question. A film of 0.05 mm thickness was cut from the tubings using a microtome. The IR-spectrum of this film showed the absorption bands characteristic for polyvinylchloride and for di(2-aethylhexyl)-phthalate, the latter apparently being the main constituent of the plasticizer used. Minor quantities of diaethylphthalate can, however, be present in the tubings as well. Its absorption bands might be hidden under the strong overlapping bands of di(2-aethylhexyl)-phthalate.

Preliminary results of perfusion experiments with different commercially available sets of blood tubings (Cobe Lab., Alwall-Gambro (standard system of the unit) and Travenol) have hitherto been negative as far as detection of diaethylphthalate in the perfusates is concerned.

*General Comments*

The present investigation has shown that, by perfusion with a salt solution, a phthalic acid ester of relatively low molecular weight could be washed out from a P.V.C. blood tubing set tested for use in haemodialysis. The ester, diaethylphthalate, could not be washed out by perfusion of three other, commercially available, types of blood tubing, some of which belonged to the unit's standard equipment. Improvement of the condition of the 3 patients described was observed on resumption of the use of the ordinary dialysis system. Patients dialysed uninterruptedly with that equipment did not show signs of hepatic disease.

Phthalic acid esters may have a toxic effect on different organs and the degree of toxicity seems to increase with decreasing molecular weight of the alcohol involved (Nematollahi, Guess & Autian, 1967). Consequently diaethylphthalate may be the compound to incriminate as the cause of the cases of hepatitis.

In one case the histological liver changes seemed to become more pronounced with reexposure for a further period of time. The course of the disease, especially that of patient 3 (S. B.) who had a flare up of the symptoms on reexposure to the test apparatus, lends heavy support for the assumption of an intoxication as the aetiology of the clinical picture. However, conclusive evidence of the theory is still lacking. The histological changes found in the liver tissue, while compatible with a diagnosis of a toxic effect upon the liver, were non-specific, and negative results of tests for Au-antigen in serum hepatitis have been reported (Laiwah, Goudie, Goldberg, Davidson & Murray, 1970).

Therefore the investigations continue in order to clarify the conditions for release of contaminants from the P.V.C. tubings to aqueous solutions as well as to blood. The hepatotoxic properties of diaethylphthalate are presently under investigation in animal experiments. A preliminary communication seems nevertheless justified because of the importance of the observations to haemodialysis units.

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B.F.Goodrich

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