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Experimental studies on environmental contamination with infected blood during haemodialysis

A REPORT BY A WORKING PARTY* SET UP BY THE MEDICAL RESEARCH COUNCIL SUBCOMMITTEE ON HEPATITIS PREVENTION IN RENAL AND ASSOCIATED UNITS

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SUMMARY

To assess the relative importance of different postulated modes of spread of hepatitis B in dialysis units, blood charged with various tracer organisms was used in simulated haemodialysis runs in four laboratories, and the resulting contamination of equipment and environment was measured semi-quantitatively.

Some airborne spread of the tracer organism occurred when tubing containing contaminated blood was needled as the 'patient' went on and came off the dialyser. Virtually no small airborne particles could be demonstrated however in simulated emergencies in which a blood line was disconnected, or even when bottles of blood were dropped on to a hard floor from a height of 2 metres.

Bacillus globigii spores from contaminated blood leaked in small numbers into the dialysing fluid through apparently intact coils. T3 phage, with a particle size of the same order as hepatitis B virus, passed in small quantities through the membrane of a Kiil dialyser from blood to dialysing fluid and also in the reverse direction when added to the header tank. A number of other dialysers were also permeable to phage.

Visual assessment of the appropriate moment for inserting the venous line into the 'patient' at the onset of dialysis was shown to be unreliable, as the displaced fluid from the end of the venous line was already contaminated before it contained visible red blood cells.

Considerable contamination of exposed surfaces and of the buttons on the proportionating unit cabinet occurred. Minor visible splashing of blood was a commonplace of the laboratory experiments and was shown to be also a common event during routine haemodialysis in two of the dialysis units taking part in the studies.

INTRODUCTION

In April 1971 a conference was convened by the Medical Research Council to consider research on hepatitis with special reference to recent outbreaks of hepatitis

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in haemodialysis and transplantation centres and to recommend what further work should be done in this field. Among the problems discussed was the difficulty of drawing up suitable codes of practice for dialysis units, given the dearth of detailed knowledge on the relative importance of various possible routes of cross-infection once hepatitis virus had been introduced. A subcommittee was set up in due course to study some of the technical aspects of dialysis unit cross-infection.

Most outbreaks of hepatitis in dialysis units have been blamed on the special equipment and procedures used. Almeida and her colleagues (1971), however, reported a small outbreak in which spread by contaminated equipment was thought unlikely. Five patients, all with their own individual Kiil dialysers, developed hepatitis 10–12 weeks after the only occasion when they had been dialysed together. On this occasion another female patient later found to be HBAg-positive had had a clotted venous line. A great deal of blood was spilt during the emergency that followed, and the nurse who dealt with this also developed hepatitis. The authors concluded that the virus had probably been spread by the air-borne route and made the point that if this was so dialysis units would have to prevent as far as possible the airborne spread of blood or serum droplets as well as avoiding skin or parenteral contact with potentially infectious blood. Marmion & Tonkin (1972) discussed the various ways in which cross-infection could occur in dialysis units and remarked that tracer experiments were needed to demonstrate the plausibility of various postulated modes of spread.

In the experiments described below, blood contaminated with various tracer organisms was subjected to a number of manipulations in an attempt to simulate dialysis unit conditions and to estimate the relative importance of different routes in the spread of cross-infection once the virus of hepatitis B had been introduced into a given unit. The experiments were done in four different laboratories.

EXPERIMENTAL METHODS AND RESULTS

EXPERIMENTS ON A SIMULATED DIALYSIS UNIT

Methods

With the help of the medical and nursing staff of the Exeter dialysis unit and of the Microbiological Research Establishment (MRE), Porton, an experimental mock-up of a patient undergoing haemodialysis was assembled in the Exeter public health laboratory. Blood containing *Bacillus globigii* spores as a tracer was used to prime the blood circuits, and the sister-in-charge of the unit performed various procedures as described below. The resulting contamination of the environment was measured by culture of the tracer organism.

The laboratory layout used for the experiments is shown in Fig. 1. Dialysing fluid diluted as for normal use was delivered from a Dylade B Fluid Supply Unit (A) to a Dylade Coil Recirculator (B) standing on a small table. The recirculation was maintained by a special pump mounted alongside. Part of the dialysing fluid was returned through the blood leak detector and then to waste. The fluid in the Recirculator was also slowly replaced by fresh fluid from the Fluid Supply Unit, the overflow from the recirculation pot also running to waste. The 'patient's'

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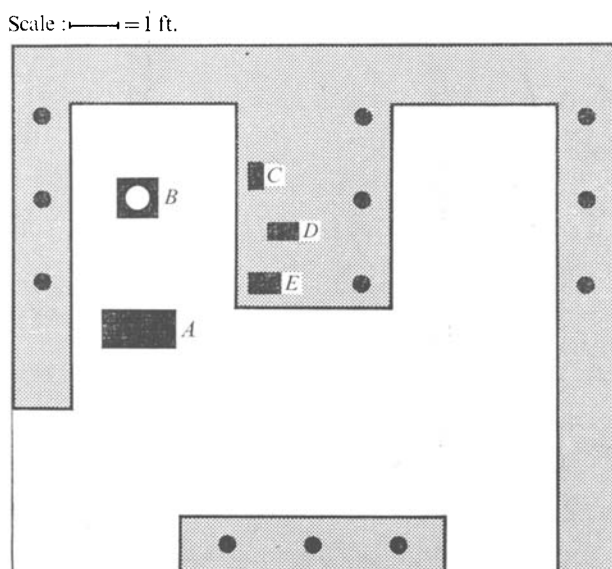

Fig. 1. Laboratory layout of Exeter experiments. *A*, Dylade B Fluid Supply Unit; *B*, Dylade Coil Recirculator; *C*, needling site of the 'patient's' shunt; *D*, slit sampler; *E*, 3-stage sampler. Settle plates were exposed during the experiments where the black circles are shown in the figure.

shunt or fistula was needled at *C*. Environmental contamination was measured with a slit sampler (*D*) and a 3-stage sampler (*E*). At the twelve black circles shown along the periphery of the laboratory and on the central bench settle plates were exposed during the experiments.

The blood and dialysing fluid circuits were set up with standard dialysis unit equipment and fittings as shown in Fig. 2. The anti-clockwise blood circuit shown on the left of the diagram represents the 'intracorporeal' circulation of the mock patient, maintained by a Watson–Marlow pump (*A*). The 'extracorporeal' blood flow was drawn off at *B*, passed through a second blood pump (*C*) and then through an Extracorporeal Europe SA coil (type EXO1 or EXO3) immersed in the Dylade Coil Recirculator (*D*). It was then returned through a bubble trap (*E*) and back into the main circulation at *F*. The narrow bore tube *G* represents the venous pressure line in routine dialysis. The blood circuits were primed with time-expired transfusion blood from the hospital blood bank, previously seeded with *B. globigii* spores and then introduced into the system at *P*, from a transfusion bottle mounted at *R*, through a blood-giving set *S*. When experiments were in progress, the pressure in the 'intracorporeal' system was maintained at 80 mmHg (10·6 kN/m.^{2}) by adjusting the blood pump rate and by varying the height of the reservoir *R*. The flow rate of the circulating blood in this part of the system was maintained at 120 ml. per minute.

In routine dialysis unit practice, blood is most likely to be spilt at the beginning or the end of dialysis, but more serious haemorrhages are not uncommon, e.g. when arterial or venous lines become disconnected. In the present series of experi-

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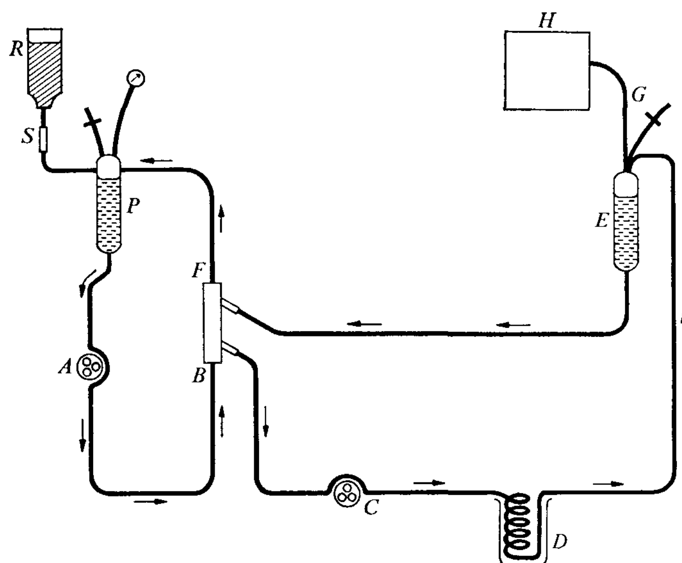

Fig. 2. Blood and dialysing circuits. The 'intracorporeal' circulation is on the left of the diagram, the 'extracorporeal' circulation on the right. *R*, Blood reservoir; *S*, blood-giving set; *P* and *E*, bubble traps; *A* and *C*, Watson–Marlow pumps; *D*, Dylade Coil Recirculator; *G*, venous pressure line. *H*, Dylade B Fluid Supply Unit. *B* and *F* are the needling points.

ments, two routine procedures and two mishaps were simulated, using blood containing between 5 and 9×10^8 spores per ml. The experiments thought least likely to give rise to massive contamination were done first. In the intervals between them, the room was aired for at least 30 min. to reduce the number of air-borne tracer cells, the circulations of blood and dialysing fluid were re-established and monitoring of the environment continued.

The four experiments, in the order in which they were done, were as follows:

I. Setting up dialysis, i.e. needling the 'patient's vein' with 15-gauge (1·8 mm. diam.) needles to set up the right-hand circuit in Fig. 2; the needles were then withdrawn and immediately re-inserted.

II. Disconnecting the 'extracorporeal' circuit from the dialyser and washing the dialyser coil through with saline.

III. A simulated burst of the dialyser coil.

IV. A simulated burst of a main blood line distal to the blood pump.

Results

In the attempts to recover the tracer organism from the laboratory environment, the main technical emphasis was laid on the detection of small airborne particles capable of being inhaled. In addition, swabs were taken at the end of the run from a number of exposed surfaces. Cultures of the dialysing fluid were also made. The findings are described below.

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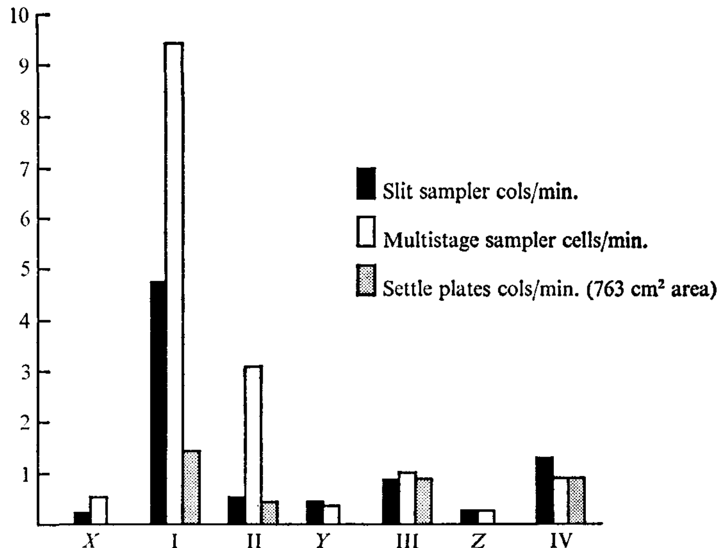

Fig. 3. Airborne spread of marker organism during four simulation experiments. I, needling the 'patient's' vein; II, disconnecting the extracorporeal circuit and washing the coil with saline; III, a simulated burst of the dialyser coil; IV, a simulated burst of a main blood line distal to the blood pump; X, Y and Z represent background contamination levels in blank experiments done in the intervals between the main experiments.

Airborne spread

The slit sampler, multistage sampler and settle plate counts are summarized in Fig. 3, in which the contamination liberated by the four manipulations in turn is shown from left to right. The degrees of contamination demonstrated are all expressed as numbers of colonies or of cells per minute. The numerical results obtained with the three methods of estimating airborne contamination are for a number of reasons not directly comparable with one another. The slit sampler, for instance, sampled 25 l. of air per minute and the multistage sampler 55 l. per minute. Again, colonies found on the slip sampler plates and on the settle plates would have arisen from particles, but those recovered from the multistage samplers used in the investigation would have grown from single cells. The number of tracer cells present in particles large enough to settle on open Petri dishes exposed in the turbulent conditions of the test room would also be greater than in particles recovered by the air samplers.

A rigid comparison between the degrees of contamination shown as a result of procedures I, II, III and IV is also unjustified for various reasons. First, the distances between the slit sampler and the points from which environmental contamination was taking place were not exactly the same in the four experiments. Secondly, as already mentioned, procedure I was done twice in succession. Thirdly, blood used in experiments I, II and IV was found to have a viable count of 5×10^8 *B. globigii* per ml., while that used in experiment III had a count of 9×10^8 per ml.

With all the caveats made above, the results summarized in Fig. 3 point strongly

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Table 1. *Particle size distribution of multistage sampler counts*

Procedure	Particle size (μm) > 6	3–6	< 3	Total count
I	270	13	2	285
II	46	0	0	46
III	10	19	1	30
IV	18	0	0	18

to a quite unexpected finding, i.e. that needling procedures gave rise to most aerial contamination. A similar result had been found in preliminary tests using blood with a much lower concentration of tracer cells. In sharp contrast, the more dramatic simulation of a coil or blood line burst caused relatively slight airborne spread. That the contamination shown in Fig. 3 could be confidently ascribed to the manipulations in progress when the air samples were being collected is given support by the low background contamination detected in the blank monitoring tests X, Y and Z done during the day and also shown in Fig. 3.

The distribution of particle sizes in samples collected by the multistage sampler during the four experimental procedures is shown in Table 1. It will be seen that most of the particles containing *B. globigii* were greater than 6 μm. in diameter.

Surface contamination

At the end of the day's run swabs were taken from various surfaces and plated out for *B. globigii*. Cultures from the mute alarm button, from the surface of the dialyser table, from the inner surface of the lid of the recirculation pot, from blood specks on the front of the Dylade Unit and from the front of Sister's plastic apron all yielded an uncountable number of colonies. The swab from the blood pump reset button yielded 133 colonies and one taken from the dialysis button 81 colonies. A swab from the skin of Sister's wrist yielded 4 colonies. None were found on cultures of her spectacles.

Culture of dialysing fluid

After experiment III, the simulated coil burst, the dialysing fluid in the recirculation pot was found to contain $2{\cdot}4 \times 10^6$ *B. globigii* per ml., corresponding to a leakage of 32 ml. of blood. Before this, however, when the coil was apparently intact, leakage had already occurred, presumably through minute faults in the coil. The numbers of *B. globigii* found after experiments I and II were equivalent to leakages of 0·07 ml. and 0·025 ml. of blood respectively. This amount of blood was below the threshold of the blood leak detector, which did not give the alarm during this time. In a later series of experiments before the mock-up was taken to pieces one out of 6 new coils showed a similar leakage of small quantities of blood into the dialysis fluid.

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Further Studies on Airborne Droplet Formation resulting from the Use of Hypodermic Needles

Methods

The airborne contamination liberated by needling tubes containing blood was later studied under more controlled conditions by two of us at MRE, Porton. In this work the 'patient' was a closed system of rubber tubing inside which citrated human blood containing *B. globigii* spores was circulated at a rate of 150 ml./min. and at a pressure of 100 mmHg (13·3 kN/m.^{2}). The tubing passed through connectors into a Porton ventilated Safety Cabinet, where it bifurcated into two parallel tubes with cross-connections of soft brown rubber as supplied with Avon Kiil blood lines, or of double rubber-over-plastic tubing from disposable blood tubing sets as supplied by Extracorporeal Medical Specialities. The various needling experiments were done on one or other of these two types of tubing, which are used in the Exeter dialysis unit. The safety cabinet was fitted with a high efficiency filter on the air inlet port and was equipped with a vacuum line to an external pump for the operation of the air sampling devices inside the cabinet. All operations inside the cabinet were carried out by an operator wearing rubber gauntlets which also served to seal the arm holes.

The air inside the safety cabinet was sampled with (*a*) a cyclone sampler concentrating the cells from 75 l. of air into 1 ml. of fluid per minute, to measure the number of *B. globigii* cells present, (*b*) an automatic slit sampler collecting 25 l./min. to measure the number of spore-carrying particles, and (*c*) a cascade impactor sampling at a rate of 17 l./min. to give an estimate of the size distribution of the airborne particles. The aggregate air flow through the three sampling devices was 117 l./min. The volume of the cabinet was 377 l. During the periods when sampling for air contamination was in progress, therefore, the time required for one air change inside the cabinet was about 3 min. Before and after each sampling period, usually of 6 min. duration, the cabinet was ventilated at a far higher rate of 3000 l./min. by means of the main ventilating fan fitted to this type of equipment. Background air samples were collected before, during and after each series of tests. To assess contamination from droplets too large to remain airborne, open Petri dishes containing tryptone agar were placed about 30 cm. on either side of the needling site during each test.

Most of the experiments were in sequences of three replicate tests of each procedure. In a typical experiment, a needle attached directly or by a short length of tubing to a syringe was inserted into the blood-filled tubing under test. The air was sampled for 6 min., the slit sampler and cascade impactor running throughout this period. The cyclone sampler collecting tubes were changed every 2 min. The sampling devices were then turned off and the cabinet ventilated for some minutes using the main extract fan. The other two needles were then inserted in turn, using a fresh section of the tubing for each needle and the same air sampling schedule. The cabinet was then ventilated again and sampling repeated during the subsequent withdrawal of each of the three needles in turn. Gauze swabs were used to cover the needles as they were withdrawn from the circulating blood.

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Table 2. *Recovery of* B. globigii *from air following insertion or removal of hypodermic needles from tubing with blood containing* 1×10^9 *spores per ml.*

Needle size (gauge)	Tubing used	Number of tests	Mean number of BG cells recovered and standard error of mean		Mean number of BG containing particles recovered and standard error of mean		Ratio of recoveries $\frac{\text{Removal}}{\text{Insertion}}$		Estimated volume of blood recovered from the air (μl)	
			Insertion	Removal	Insertion	Removal	Cells	Particles	Insertion	Remova
15	A	7	550 ± 220	2500 ± 750	260 ± 100	900 ± 170	4·5	3·5	0·0006	0·003
21	A	3	150 ± 50	900 ± 600	85*	500 ± 350	6·0	5·9	0·0002	0·001
25	A	3	< 20	< 20	< 20	< 20	—	—	< 0·00002	< 0·00002
23	B	6	< 20	< 20	< 20	< 20	—	—	< 0·00002	< 0·00002
25	B	3	< 20	< 20	< 20	< 20	—	—	< 0·00002	< 0·00002

* Only 2 results available: one sample spoilt.
A = Avon Kiil blood lines. Rubber 6·5 mm. O.D., 3·0 mm. I.D.
B = Extracorporeal Medical Specialities. Double tubing. Outer tube rubber 10 mm. O.D., 6·5 mm. I.D. over plastic inner tube 6·5 mm. O.D. 4·5 mm. I.D.

The extent of air contamination associated with each experiment was calculated from the cyclone sampler and slit sampler counts, taking into account the air volumes sampled by the different samplers and the clearance resulting from the withdrawal of air from the cabinet for sampling. Allowance had to be made also for the fact that in different experiments the viable counts of *B. globigii* in the batches of blood used ranged between 0·7 and $1{\cdot}9 \times 10^9$ per ml. To simplify presentation, counts obtained have been adjusted to what they would have been had the blood liberated into the air of the cabinet in a given experiment contained 1×10^9 organisms per ml rather than the viable count actually obtained.

Results

The results obtained with needles of four different gauges have been brought together in Table 2. The arithmetic means of the corrected cell and particle recoveries are shown in the table and the standard errors of the means. From the viable spore recoveries, estimates of the volumes of blood recovered from the air were calculated and are shown in the right-hand columns. The table shows maximum recovery of *B. globigii* from the air of the cabinet when the needles of widest bore, 15-gauge (1·8 mm. diam.), were used. A lower recovery of about a third as many was recorded with needles of 21-gauge (0·8 mm. diam.). With finer needles of 23-gauge (0·6 mm. diam.) or 25-gauge (0·5 mm. diam.), tested with either single or double tubing, the small numbers of tracer cells recovered could not be distinguished from the persistent low level background inside the cabinet.

With both 15- and 21-gauge needles, recovery of the tracer organism was on average five times greater following withdrawal of the needle from the tubing than after its insertion. This was not unexpected, as however carefully needles were withdrawn from the tubing some vibration of the needle tip was likely to occur. The slit sampler counts obtained during the sampling periods when six 15-gauge needles were inserted and then withdrawn are shown grapically in Fig. 4.

In only one series of tests with 15-gauge needles were enough viable organisms

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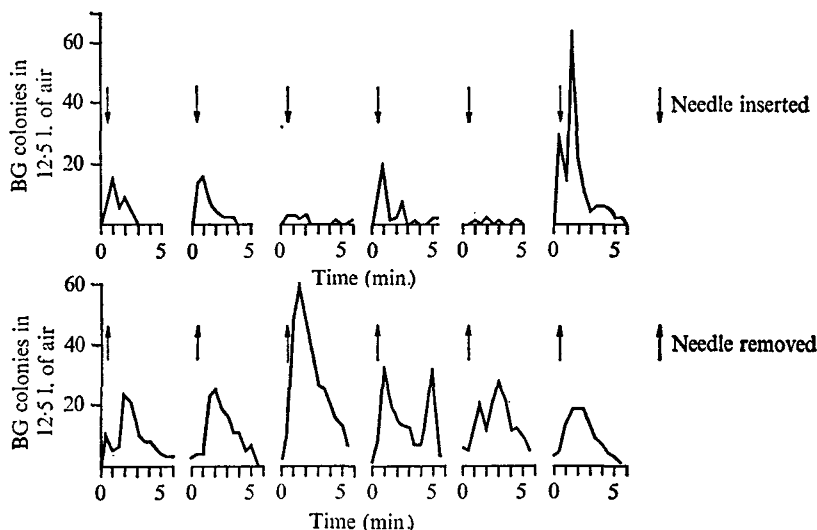

Fig. 4. Slit sampler counts during the 5 min. periods after insertion and after removal of 15-gauge needles, showing the greater airborne spread associated with withdrawal of the needles.

collected by the cascade impactor to give a reliable estimate of the size distribution of *B. globigii*-containing particles in the air of the test cabinet. On that occasion the size distribution was as follows: 6–20 μm., 40%; 2·2–6 μm., 40%; 1–3 μm., 14%; 0·5–1·5 μm., 6%. Plotting these recoveries gave an estimated mass median diameter of 11 μm.

With 15-gauge needles and on one occasion with a 21-gauge needle, drops of blood were seen to impinge on the floor and walls of the cabinet. With these needle sizes open Petri dishes exposed on the floor of the cabinet were invariably positive for *B. globigii* after incubation, although only in one test was macroscopic blood visible on the plate. Given the turbulent conditions inside the cabinet and the short 6 min. exposure time of the open Petri dishes, the droplets giving rise to colonies when the plates were incubated must have been quite large.

Tracer Experiments with T3 Bacteriophage

One of the uncertainties of extrapolating from tracer experiments with bacterial spores to the assessment of real life hepatitis risks derives from the disparity in size between the bacterial tracer and the virus. On this account, a number of experiments were done at East Birmingham Hospital using a T3 phage which has a particle size of 58 nm. It is widely believed that the infectious unit of hepatitis B in the blood is at least 35 nm. and probably about 40–50 nm. in diameter.

The first experiments centred on the permeability of dialysis membranes to the phage. A Watson–Marlow Kiil dialyser and a Lucas Proportionating and Monitoring Unit were set up as though for dialysis. The 'patient' was a litre of T3 phage dilution in saline broth or blood, containing about 10^7 plaque forming units (p.f.u.) per ml. The blood circulation was maintained with a Watson–Marlow pump.

Thirteen dialyses were run, 10 with a saline broth 'patient' and 3 with a mixture

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of time-expired heparinized human blood 33 % in saline. Each dialysis was continued for from 2 to 4 hr., with a blood flow of 150 ml./min. and a dialysing fluid flow of 500 ml./min. in the opposite direction. The 'venous' and dialysing fluid pressures were +40 mmHg (+5·3 kN/m.²) and −40 mmHg (−5·3 kN/m.²) respectively. Samples of the dialysing fluid effluent were taken before, during and after dialysis and from the 'patient' before and at the end of dialysis. In 4 out of 10 runs with a saline broth 'patient', phage was detected in the dialysing fluid. The highest count was 200 p.f.u./ml.; in the other three, it was less than 50 p.f.u./ml. No phage was found in the dialysing fluid during the three runs with blood saline.

Further experiments were done to see whether phage added to the dialysing fluid could cross the dialysis membrane in the opposite direction against a head of 80 mmHg (10·6 kN/m²) pressure, and infect the 'patient'. In 1 out of 3 experiments, a low concentration of phage – less than 10 p.f.u./ml. – appeared in the 'patient' circuit from a dialysing fluid that contained 10^3–10^6 p.f.u./ml.

In later experiments, a number of other commonly used dialysers were tested for the passage of T3 phage from the 'patient' into the dialysing fluid. The Travenol Ultra Flow 100 and the Avon 'Nephravon' R 71 were both permeable to phage. Negative results were obtained with the Cordis–Dow Model No. 4 and the Travenol Ultra Flow II. The Cordis–Dow Model No. 2 was tested twice, and gave one positive and one negative result.

With no prior knowledge of the Exeter findings described earlier, two experiments of a different kind were done to assess the airborne dissemination of infective material to be expected as a result of gross spillage of human blood. In both experiments T3 phage was added to 750 ml. bottles of time-expired transfusion blood to a titre of about 10^8 p.f.u./ml. The bungs and aluminium caps were replaced and the bottles then dropped from a height of about 2 m. on to a hard floor of vinyl tiles laid on concrete and broken. Petri dishes containing nutrient agar flooded with the phage-propagating strain of *E. coli* were exposed on the floor at distances of 1–10 m. from the impact point and also at various sites above ground level. A slit sampler sampling 62 l. of air per minute was placed 3 m. from the impact point and 1 m. above ground level.

In preliminary experiments to check the sensitivity of detection of airborne phage, 0·04 ml. of heparinized blood containing 2×10^8 p.f.u./ml. was sprayed by means of a nebulizer into the air of the test room, which was 68 m.³ in size. Phage was detected on all the exposed settle plates except one that was 30 cm. below ceiling level; it was also detected by the slit sampler. When on the other hand the two bottles containing phage-contaminated blood were dropped from a height of 2 m. on to the floor and broken, no phage was detected in the air samples or on settle plates not visibly splashed with blood. Only visibly contaminated plates showed the presence of T3 phage. These experiments with phage thus confirmed the Exeter findings that human blood does not readily form an aerosol under dialysis unit conditions. Comparable to the Birmingham work on phage dissemination was one test at Exeter in which a transfusion bottle of blood containing 5×10^8 spores/ml. was shaken vigorously to produce foam and then exposed to a slit sampler. The open neck of the bottle was held about 3 cm. from the air intake

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to the sampler. During a 7 min. period in which bubbles were to be seen bursting in the blood foam only 8 colonies of the tracer organism were recovered.

In the Birmingham bottle-dropping experiments the pattern of spread of the spilt blood was capricious. A cine-film taken at 64 frames per second with an effective exposure of 1/128 sec. showed that the bottle bounced on impact. The bottle neck and top reached a final position on the floor some 30 cm. away from the point of impact. Blood could be seen on the ground over a circle of some 2 m. radius but had not spread uniformly. The shape of the drops, in long thin lines spreading from the centre, indicated a considerable velocity of propulsion at a low angle of incidence. This pattern of spread may however have been determined in part by the surface properties of the vinyl tiles upon which the bottles were dropped. During one of the two experiments, blotting paper screens were also placed vertically 60 and 120 cm. from the point of impact so as to measure the height above ground to which splashing occurred. The distribution of splashes on the 60 cm. screen indicated that circular splashes to a height of 21 cm. had been propelled from the initial impact point and that oval or elongated splashes to a height of 47 cm. had been emitted from the final resting place of the bottle neck and top. In the same experiment, the vertical screen placed at 120 cm. was unmarked, although splashes to a greater distance could be seen on the floor in other directions.

In the course of later discussion of these findings the suggestion was made that T3 phage was a delicate one and that a more resistant tracer, T1 phage for instance, should have been used. To test this, 6 experiments were done by spraying a mixture of T1 and T3 phages into the air of the test room and estimating the comparative decay rates with settle plates. Under the conditions of these experiments, T3 phage was found to be as stable as T1.

Experiments with a Gram-negative Tracer Organism

In earlier work at Withington Hospital, Manchester, Jones and his colleagues (1972) found that during 1968 their dialysis unit became colonized with *Enterobacter aerogenes*, which was regularly isolated from dialyser effluents. During the following year, this organism was isolated from 24 % of blood cultures taken from patients suffering from pyrogenic reactions. In addition, all patients dialysed in the unit during this period were found to have O and H antibodies to the same organism, which were interpreted as a response to antigenic experience of whole bacteria that had penetrated the Cuprophane membranes, probably through small tears. As part of the present study, the same workers made a number of observations on the environmental contamination to be expected under dialysis unit conditions when blood containing *Escherichia coli* was dialysed through a Kiil kidney used in conjunction with a Dylade Supply Unit. The main findings are summarized below.

(1) Defibrinated horse blood containing 7×10^6 *E. coli* per ml. was pumped through a warmed Kiil dialyser in exactly the same manner in which the arterial line from a patient is connected up at the start of dialysis. As the saline contained in the blood compartments of the dialyser was displaced by the infected blood, samples were collected from the end of the venous line for culture. In accordance

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with established routine, a member of the unit staff clamped the venous line when red blood cells were judged visually to have reached a point 45 cm. from the end of the line. Cultures showed that this would have been too late to prevent contamination with the tracer organism of whatever receptacle was used in everyday routine practice to collect the displaced saline solution prior to connecting up the venous line to the 'patient'. Visual inspection of the blood flow in the venous line provided the probable explanation. Examination of the advancing line of red cells showed that the flow along the central axis was well ahead of that at the periphery, forming a very finely drawn-out spearhead the front of which was invisible to the naked eye. This finding suggested that the venous line should be clamped as soon as blood had been seen to enter the bubble trap proximal to the end of the line.

(2) To simulate venepuncture more realistically than in the Exeter and Porton studies, a Meltec Artificial Arm, which is covered by a plastic 'skin', was set up and the 'blood vessels' in it filled with broth or defibrinated horse blood containing 10^8–10^9 *E. coli* per ml. Slit sampler runs were made alongside in the course of a number of venepuncture manipulations. In preliminary experiments with static or circulating broth containing 10^8 *E. coli* per ml. at pressures of 70–150 mmHg (9·3–19·9 kN/m.^{2}), no evidence of airborne dissemination of the tracer organism as a result of 'venepuncture' could be found and settle plates remained sterile. In a later experiment with infected blood in which slit sampler plates remained sterile, two settle plates placed 27 cm. away from the venepuncture site showed 2 and 5 colonies of the tracer organism respectively, and a third settle plate on a bench beneath the artificial arm showed 24 blood splashes.

Tests with needles of varying bore were done on the Artificial Arm using a higher concentration of the indicator organism. 200 ml. of defibrinated horse blood containing 10^9 *E. coli* per ml. was circulated by means of a pump at a pressure of 20 mmHg (2·6 kN/m.^{2}), using an Avon blood line with standard rubber inserts as the external circuit. Venepunctures were done with needles of gauge 14, 16, 21 and 25. In this experiment the only needle that gave rise to airborne spread of infective material was the 25-gauge (0·5 mm. diam.), which vibrated visibly when used. When this needle was inserted and removed 10 times in succession, the 1 min. counts on serial slit sampler plates sampling 1 cu.ft./min. were 1, 25, 8 and 2 respectively.

(3) In a final experiment, a Kiil dialyser and a Dylade Supply Unit were set up as though for patient dialysis. A bottle of defibrinated horse blood containing the indicator organism was attached by two 2 ft. lengths of plastic tubing with rubber inserts to arterial and venous lines through butterfly needles, and dialysis was run for 30 min. at a pressure of 30 mmHg (4 kN/m.^{2}). All slit sampler plates run before, during and after dialysis remained sterile. The viable count of *E. coli* in the blood used was however only 5×10^7 organisms per ml. The negative findings in this experiment are therefore not surprising.

SPLASHING OF BLOOD IN THE COURSE OF ROUTINE HAEMODIALYSIS

All the experiments described above indicate that blood, presumably because of its rheological properties, does not readily form small airborne droplets under

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Table 3. *Counts of blood splashes of different sizes at insertion and removal of needle in 22 dialyses on 10 patients*

	Insertion of needle Diameter of splash (mm.)			Removal of needle Diameter of splash (mm.)		
Patient	> 10	1–10	< 1	> 10	1–10	< 1
P	0	0	0	0	1	0
	0	9	0	0	2	0
K	3	19	19	2	7	11
	7	9	26	0	2	4
H	1	19	Smudges	0	0	0
	1	9	3	0	1	8
S	0	8	12	1	2	0
	0	0	0	0	7	0
E	1	0	1	0	1	1
	1	10	9	0	5	18
M	3	5	0	0	13	52
	0	9	5	0	0	0
GN	0	1	11	0	0	0
	0	10	12	0	0	0
	1	2	1	4	41	68
	0	1	0	0	0	0
GY	0	3	5	0	7	0
	0	0	0	1	19	4
W	9	35	41	0	1	4
	6	19	60	0	0	0
GL	6	17	10	0	12	12
	1	15	12	0	6	7
Totals	40	200	227	8	127	189

dialysis unit conditions. Minor splashing, by contrast, was often observed. This prompted speculation on the extent to which splashing of blood might occur in routine dialysis unit practice when patients went on and off the dialyser. In the Withington Hospital Unit all patients dialysed in the course of one week had large filter paper sheets 68 × 58 cm. placed under the arm at the beginning and at the end of dialysis. Blood splashes on the paper were counted and graded in various sizes, and the results are tabulated in Table 3. Two points emerge clearly from the table. First, minor splashing of blood was a frequent event. Secondly, the distribution of counts on individual patients tends to show them as either very low or high. In practical terms, the insertion or the removal of needles sometimes goes straightforwardly and virtually no blood is split. On other occasions, perhaps because the needle misses the vein, considerable splashing occurs. The patient GN, for instance, had total splash counts of 12, 22, 117 and 1 in successive dialyses. Similar findings were recorded in a parallel study in Exeter. In five successive dialyses, for instance, splash counts on one Exeter patient were 48, 0, 3, 0 and 1. On another patient the splash counts in two successive dialyses were 45, 0. Even under the carefully controlled conditions of the Porton experiments the results of replicate experiments

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done at one session showed considerable variation. It is perhaps not surprising, therefore, that in the daily routine of dialysis unit practice the manoeuvres required to put a patient on and off dialysis, straightforward though they may seem, should also give rise to a very variable amount of blood splashing.

DISCUSSION

In 1968, a PHLS working party made various recommendations aimed at reducing the risks of hepatitis in dialysis units. At that time, no method for the identification of dangerous carriers of hepatitis B virus was known. The recommendations made were therefore essentially recipes for a good standard of hygiene based on experience of other types of hospital cross-infection. Since 1970, systematic screening of dialysis unit patients and staff for HB (Australia) antigen has greatly strengthened hepatitis prevention in these units. This is evident from the report of Polakoff, Cossart & Tillett (1972), who found that when the first antigen positive patients detected in five different dialysis units were isolated no further spread of infection occurred. The problem of dialysing the antigen positive patient remains, however, as a legacy of the years before screening tests were available. The study of environmental contamination by infected blood is also relevant to the problem of cross-infection when patients with unsuspected hepatitis B are admitted to general hospital wards.

The experiments recorded in this paper were planned mainly with a view to assessing the relative importance of the various possible channels of cross-infection in dialysis units so as to improve codes of practice. The working conditions of the average dialysis unit were simulated as closely as possible, and some of the tests were in fact done within dialysis units. The consistency of our findings with different indicator organisms in different laboratories suggests that the results are valid and applicable to the dialysis of patients.

The most striking negative finding was a uniform failure to detect airborne spread of infected blood when dramatic emergencies causing gross surface contamination were simulated and even when bottles of blood were shattered on the floor from a height of 2 m. When contaminated blood was discharged through a nebulizer, an indicator phage was readily recovered from the air of all parts of the test room. Under dialysis unit conditions, by contrast, it would seem that presumably because of its viscosity blood does not readily form an aerosol. The present experiments do not therefore give support to the hypothesis of an airborne spread proposed by Almeida and her colleagues (1971) as the explanation of the outbreak they described.

An unexpected finding of the Exeter work was that although major spilling of blood did not produce measurable numbers of small airborne particles, the insertion and removal of needles from tubing containing blood did give rise to appreciable numbers of airborne particles in the size range capable of being inhaled by man. This was confirmed by the more rigorously controlled experiments at MRE, Porton which showed that airborne spread of blood was particularly likely to occur when a needle was withdrawn rather than when it was inserted. In the Porton work,

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airborne spread took place only with needles of 15-gauge (1·8 mm. diam.) and to a lesser extent with 21-gauge (0·8 mm. diam.) but not with finer sizes. In Manchester, on the other hand, aerosol production was found only with a 25-gauge (0·5 mm. diam.) needle that vibrated when used. The quantity of airborne spread from needles may depend partly on their bore and length, partly on how much they vibrate in use and partly on the care exercised by the operator. So far as dialysis unit practice is concerned, these findings reinforce the commonly held view that the main risk to staff is incurred when patients go on or come off dialysis and that at these times protective clothing should be worn.

The Advisory Group on Hepatitis and the Treatment of Chronic Renal Failure (Report, 1972) stressed that a particular objective of research into artificial kidney design should be to develop an effective means of isolating the proportionating machines and monitors from the possibility of contamination by blood. The present studies showed that a phage of particle size similar to that of hepatitis B virus could traverse dialysis membranes in either direction. In the Exeter work *B. globigii* spores also made their way through apparently intact coils. Whether leakage occurred through minute faults in the membranes or through interstices in intact membranes or coils cannot be stated, but of course a dialysis membrane of any kind may burst while in use. The permeability of membranes to infective agents suggests that the entire fluid pathway downstream of the dialyser should be disposable, especially when the machine is to be used on antigen-positive patients. This could be done by replacing the conventional effluent pump with one of a roller type, by the use of a disposable blood leak detector cell and in some machines by resiting the flowmeter. The Manchester work also draws attention to another flaw in dialysis unit procedure, i.e. the possibility of effluent from the end of the venous line being contaminated before the line is inserted into the patient at the onset of dialysis, with resulting contamination of the receptacle into which the waste saline has been collected.

Perhaps most important of all from the standpoint of day-to-day routine procedures in dialysis units was the evidence from these studies of the gross contamination of all adjacent surfaces that occurred during the manipulations associated with dialysis. This has several implications as regards the design of equipment and the maintenance of unit hygiene. The considerable contamination demonstrated on the buttons on the front of the Dylade Supply Unit prompts the question whether buttons should be redesigned so as to take small disposable covers. Alternatively, switch mechanisms might be covered by a membrane flush with the surface of the cabinet, which could then be swabbed down with disinfectant after use. The frequency of blood splashing came as a surprise even to dialysis unit staff and was also commented on during the mock-up experiments in Exeter and Porton. This together with the heavy positive cultures obtained from surface swabs points the need for greater attention by nursing auxiliaries to the maintenance of scrupulous cleanliness of the patient's environment, with frequent swabbing down of all surfaces preferably with a hypochlorite solution.

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