

## Bacterial Contamination of Dialysing Fluid of Artificial Kidney

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During haemodialysis with the Kolff twin-coil artificial kidney the patient's blood is passed through "cellophane" tubing which is immersed in a dialysing bath. This holds 100 litres of fluid, with an electrolytic composition similar to that of the normal plasma, with an addition of 0.88% glucose. During haemodialysis, urea and other nitrogenous substances appear in increasing quantities. The bath is kept at a constant temperature of 37° C. and it was formerly our practice to change the dialysing fluid every three hours. (In the light of experience, however, we now change the bath at much shorter intervals.)

In our early endeavours with this equipment we were disturbed to note an increasing turbidity of the fluid in the bath as dialysis progressed. Knowing that the contents of the dialysing bath constituted an ideal culture medium for some bacterial species, we suspected that the turbidity might be the result of bacterial growth.

This led us to make a bacteriological investigation of the contents of the bath. The result of this we are now presenting.

The equipment had been in clinical use for approximately two years at the time this inquiry was instituted, and it was customary to subject the equipment only to routine cleaning with soap, detergents, or mild abrasives.

### Bacterial Contamination of Dialysing Fluid

#### Methods

During dialysis 1-ml. samples of the dialysing fluid were examined bacteriologically by means of a viable count using a technique similar to that described by Miles *et al.* (1938). After incubation at 37° C. for 24 hours at least three drops of a suitable dilution on nutrient agar were used to calculate the number of organisms.

Moistened swabs were employed to examine various parts of the dialysing bath and reservoir tank in which the second and third changes of solution were prepared. These swabs were taken after the tank had been cleaned and were plated on nutrient agar and incubated at 37° C. for 24 hours.

#### Results

A typical result of the viable counts during a dialysis is shown in Fig. 1. The total count plotted against the time of sampling showed two precipitous falls corresponding to the times when the bath fluid was changed. During the changing of the bath the whole contents cannot be removed by the pump, so that each new bath is necessarily inoculated from its predecessor. Since there were 60,000 organisms per ml. in the first bath of a dialysis it was also evident that this initial preparation was contaminated.

The same four bacterial species were identified in the bath fluid at the beginning of each dialysis examined. They were *Pseudomonas pyocyanea*, *Escherichia coli*, *Pseudomonas stutzeri*, and *Achromobacter* species (sp.). As the dialysis proceeded *Ps. pyocyanea* and *Achromobacter* sp. multiplied far more rapidly than the other two organisms; at the end of a dialysis there were usually about  $10^7$  *Achromobacter* sp. and  $10^6$

*Ps. pyocyanea* per ml. and neither of the other two organisms was isolated.

Cultures of the swabs taken from the metallic surface of the dialysing bath following the routine cleaning procedure revealed a scanty growth of each of the four organisms found in the dialysing fluid. However, swabs taken from the plastic tubing connecting the bath with the drainage and circulating pumps revealed many of each of the four organisms.

None of these organisms were obtained from the reservoir tank used to make up the second and third batches of solution.

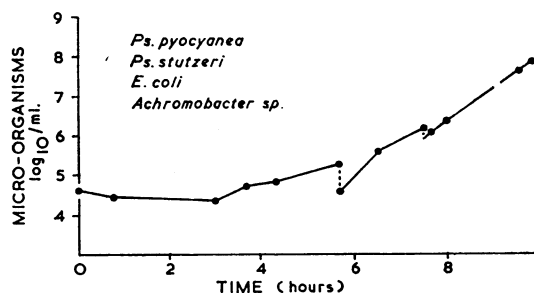


FIG. 1.—Result of viable counts made during a dialysis. The bath received no disinfectant treatment during dialyses. The sudden lowering of the viable count corresponds with the changes of fluid during the dialysis.

As unsterile tap-water, passing through the same tubing, was used to make up all batches of solution, including the two prepared in the reservoir tank, the tap-water was excluded as the source of the contamination. The dialysing tank itself was therefore regarded as the source of the contamination.

### Effect of Bacteria on Composition of Dialysing Fluid

#### Methods

After two hours' use of three 100-litre preparations of dialysing fluid, six 400-ml. samples from each of two batches and two 400-ml. samples from the third batch were incubated at 37° C. for 20 hours. As controls a filtered preparation containing urea but no organisms, and a freshly prepared medium of similar composition to the dialysing fluid which had not been dialysed against a patient, were selected. To 1 litre of this latter solution 20 mg. of urea per 100 ml. was added and to another litre 50 mg. of urea per 100 ml. A total of 4 litres of this synthetic bath were inoculated with a concentration of organisms similar to those found in the normal dialysing fluid.

Viable counts, urea and glucose estimations, and Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, and HCO<sub>3</sub><sup>-</sup> concentrations were determined at the beginning and end of the incubation period.

#### Results

Typical results obtained from this investigation are shown in the Table. The growth of bacteria was found to produce a lowering of the urea and glucose concentrations, but there was no alteration in the Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, and HCO<sub>3</sub><sup>-</sup> except in one instance where the bicarbonate was reduced from 29 to

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21 mEq/l. The synthetic medium was not able to support any marked growth of the bacteria unless urea was added, and even then the growth was poor compared with that in the solutions obtained after dialyses against a patient.

*Effect of Bacterial Growth on Composition of the Dialysing Fluid*

Type of Fluid	Time of Sampling (Hours)	Composition						Viable Counts (orgs./ml.)
		Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Urea	Glucose	
		mEq/l.			mg./100 ml.			
Dialysis fluid after use	0	134	5.4	102	29	50	820	4.5 × 10 <sup>8</sup>
	20	136	5.4	107	30	35	692	1.7 × 10 <sup>9</sup>
Sterile dialysis fluid after use	0	134	4.5	103	33	50	800	0
	20	138	4.6	105	34	52	780	0
Unused dialysis fluid plus urea	0	130	5.2	112	34	20	750	7.5 × 10 <sup>4</sup>
	20	146	5.2	113	34	10	740	5 × 10 <sup>6</sup>
Unused dialysis fluid without urea	0	130	5.2	112	34	0	750	9 × 10 <sup>4</sup>
	20	140	5.3	114	34	0	720	8 × 10 <sup>4</sup>

**Passage of Bacterial Substances Across the Cellophane Membrane**

**Cultures of Blood During Haemodialysis**

**Methods**

Blood cultures were prepared from samples collected at the end of 120 dialyses and in many instances from two other samples collected during the dialysis. Five millilitres of blood was removed from the external circuit, usually after the blood had passed through the coil, but 10 samples were taken before the blood entered the coil.

Three millilitres of the blood was inoculated into 40 ml. of Hartley's tryptic broth, 1 ml. to Wright's glucose broth, and 1 ml. to Colebrook's cooked-meat medium. These were incubated aerobically at 37° C. and examined for growth at 1, 2, 7, and 14 days.

**Results**

Four patients with septicaemia were submitted to haemodialysis and in all of them the responsible organism was found in each culture made during these dialyses. However, from the 300 blood cultures made on 52 patients with initially sterile blood during 116 dialyses there was no evidence that bacteria from the dialysing bath passed into the patients' blood-stream. There was no difference in the results of 10 cultures made before and after the blood passed through the coil.

**In-vitro Investigations**

**Methods**

Tubular cellophane used in making the coils for dialysis was cut into 30- to 40-cm. lengths. The two ends were attached to polyvinyl tubing of a size which could be adjusted to a hypodermic syringe nozzle. Both ends were plugged with cotton-wool, and these bags were then sealed in plastic bags and sterilized by gamma-irradiation. Fig. 2 shows the bag immersed in a test system to be described. The passage of various substances, selected for their ease of detection, was determined as described below, using the prepared cellophane bags.

(a) *Pyocyanin*.—A culture containing visible pyocyanin was prepared by incubation of *Ps. pyocyanea* in 100 ml. of Hartley's tryptic broth at 37° C. for 24 hours. A sterilized bag was immersed in the culture and 10 ml. of sterile Hartley's tryptic broth, diluted 1/5 in sterile water, was introduced by a syringe into the bag. After four hours' incubation at 37° C. the inside fluid was removed by suction from a syringe and examined for

pyocyanin by extraction with chloroform, the presence of pyocyanin in the chloroform fraction giving a blue-green colour.

(b) *Staphylococcus Aureus Haemolysin*.—A preparation of *Staph. aureus* toxin, which haemolysed a 5% suspension of washed rabbit red blood cells at a dilution of 1/200 in five minutes and at 1/2,000 in five hours, was selected. Samples of undiluted toxin and a 1/2 dilution were placed inside the test bags and were immersed in 100 ml. of saline and the systems left at room temperature for two days. Controls containing only saline within the bag were also prepared. The presence of the haemolysin was detected visually after the outside saline solution was incubated at 37° C. for 18 hours with an equal volume of 5% washed rabbit red cells.

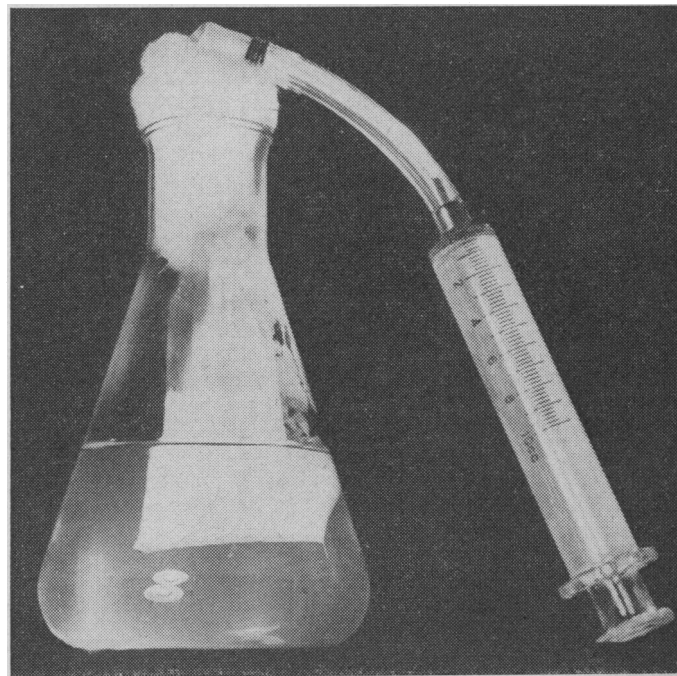


FIG. 2.—Cellophane bag system used for testing the passage of substances across the membrane.

(c) *Indole*.—Sterile bags containing 10 ml. of half-strength peptone water were immersed in 50-ml. cultures of *E. coli* in peptone water which had been incubated at 37° C. for 24 hours and contained indole. After 5 and 24 hours' immersion a portion of the inside solution was removed and the indole extracted with xylol. The presence of a pink coloration on the addition of the indole reagent was employed to detect indole.

(d) *Acetyl-methyl Carbinol*.—Cellophane bags containing 10 ml. of sterile glucose phosphate peptone water, diluted one in two in water, were immersed in 50-ml. cultures of *Aerobacter aerogenes* which had been incubated in glucose phosphate peptone water for four days at 37° C. After 5 and 24 hours a portion of the inside solution was tested for the presence of acetyl-methyl carbinol by the addition of 40% KOH and creatine.

(e) *Chlorhexidine*.—Bags containing 20 ml. of brain-heart infusion broth, inoculated with *Staph. aureus*, were immersed in 200-ml. portions of 1/200 chlorhexidine in brain-heart broth. A bag similarly inoculated, immersed in 200 ml. of sterile broth without chlorhexidine, served as the control. Each of the above tests was repeated at least three times.

(f) *Urease*.—A non-sterile preparation of urease was obtained by using tablets each of which was stated by the manufacturers (B.D.H.) to hydrolyse 160 mg. of urea in three hours at 15° C. Two tablets were ground in 20 ml. of distilled water. The urease suspension was placed inside a bag which was immersed in 200 ml. of a sterile solution containing 100 mg. of urea per 100 ml. A control was prepared by immersing bags

containing only distilled water in similar urea solutions. After incubation at 37° C. for 24 hours the urea in the outside solutions was estimated.

## Results

(a) *Pyocyanin* was detected on the other side of the membrane after four hours' incubation.

(b) When *Staph. aureus* toxin was diluted one in two the outside solution did not haemolyse rabbit red cells, but undiluted toxin appeared to pass across the membrane, since the outside solution from this latter test caused haemolysis in 18 hours at 37° C.

(c, d) Both indole and *acetyl-methyl carbinol* passed across the membrane in five hours.

(e) When *chlorhexidine* was present in the outside solution the inside culture remained sterile although it had been inoculated with *Staph. aureus*. The control system contained 10<sup>8</sup> organisms per ml. in the inside bag.

(f) *Urease*.—There was a 50% reduction in the urea concentration after 24 hours' incubation. The controls showed no such reduction.

## Discussion

Since the dialysing fluid is a synthetic medium of similar composition to plasma it is not surprising that bacteria with less fastidious growth requirements have been found in the dialysing fluid. Sherris *et al.* (1961) have reported a similar problem with contamination by *Pseudomonas* and *Aerobacter* species. The dialysing bath itself and perhaps the less accessible pumps and connecting tubing were found to be the chief source of contamination in this present investigation and that of Sherris *et al.* (1961).

However, in contrast to the findings of Scribner and Caner (1960), and Sherris *et al.* (1961) there were no instances of transient bacteraemia caused by the bacteria found in the dialysing fluid. Since wet cellophane has a pore radius of 2.5  $\mu$ , similar to that of the capillaries in the glomerular membrane, formed elements of the blood, plasma proteins, protein-bound substances, and bacteria are not expected to pass across the intact membrane (Marshall, 1961).

The study of the effect of the bacteria on the composition has shown that estimations of urea in the dialysis bath after use would not be an accurate guide to the total amount of urea removed. However, the progress of a dialysis is usually followed by an assessment of urea in the blood and not by the urea values of the dialysis fluid. In this investigation the organisms found in our dialysing bath were used; other organisms could yield different results. Scribner *et al.* (1960) attributed the reduction of bicarbonate to zero during one dialysis to bacterial contamination. With the exception of the one instance quoted in the results there was no other evidence of alteration of the ionic concentrations.

Although the bacteria themselves are unable to pass across the cellophane membrane the metabolism of the bacteria may result in the production of smaller toxic molecules which may reach the patient's blood-stream. However, no clinical symptoms could be attributable to such a hypothesis. For this reason an *in vitro* system was established in an attempt to investigate this theory.

The size of the molecule is of major importance in determining which substances will pass across the membrane. Enzymes and endotoxin molecules are large in comparison with most of the substances tested but small in comparison with a red blood cell, which is the criterion of integrity of the cellophane. The substances selected for testing were of varying molecular weights, but it was not the purpose of this study to determine the size of molecules which may pass across the

membrane. A more detailed analysis of the actual presence of bacterial products in the bath while in use and the possible passage of these substances into the patient, although desirable, was not possible.

With the artificial test system it was shown that some bacterial products may pass across the membrane, and, although most of these would not be present in a culture of *Ps. pyocyanea*, *E. coli*, *Achromobacter* sp., or *Ps. stutzeri* in a medium with the composition of the dialysing fluid, these findings are thought to represent the potential danger of contaminants to a patient. Endotoxins of Gram-negative bacteria particularly come to mind but were not studied because of technical difficulties in detecting them.

When the dialysing fluid is heavily contaminated with bacteria, and in particular *Ps. pyocyanea* towards the end of a dialysis, there is considerable aerial contamination. It is important to realize the potential pathogenicity of these organisms, especially when one remembers how readily these may be spread through a ward. Moreover, the strains of *Ps. pyocyanea* and *Ps. stutzeri* which we encountered were resistant to penicillin, tetracyclines, chloramphenicol, nitrofurantoin, and kanamycin but sensitive to streptomycin. However, with the single exception of a *Ps. stutzeri* infection in a wound after haemodialysis, there was no clinical evidence of infection in any other patients after haemodialysis by these particular organisms.

From the above evidence it is considered necessary to eradicate this source of contamination of the patient. A study of the problem is being made in this department, and at the present time the use of chlorhexidine after each dialysis has proved to be the most effective method of disinfection, the bath fluid remaining virtually sterile during the next dialysis. The technique used has been to add 6–8 litres of 1/250 dilution of chlorhexidine at 50–60° C. to the emptied tank for half an hour after each dialysis. This results in immersion of the pumps and tubing but the main tank is only moistened with the solution.

However, this is not thought to be the final answer to this problem. By nature of the composition of the fluid and the size of the container it would be necessary to have a continuous sterilizing procedure operating throughout the dialysis to maintain sterility of the fluid. At the present time the potential hazard of this 100 litres of bacterial culture should be realized and a disinfection procedure such as the one described above should be used.

## Summary and Conclusions

The dialysing fluid of the twin-coil artificial kidney was found to be heavily contaminated with bacteria.

These bacteria were not detected in the blood of patients dialysed against the contaminated solution.

The growth of the bacteria was shown to be associated with a reduction in the urea and glucose concentration.

Pyocyanin, indole, acetyl-methyl carbinol, *Staph. aureus* haemolysin, urease, and chlorhexidine were shown *in vitro* to pass across the cellophane membrane used in the artificial kidney.

Such contamination is of potential danger to the patient and should be eradicated. At the present time no completely satisfactory method is available.

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