The value of long-term prophylaxis lies in preventing reinfection of the bladder, with the accompanying risk of ascending infection of the kidney while the vesicoureteric junction remains incompetent.⁹ Both co-trimoxazole and nitrofurantoin are satisfactory prophylactic drugs^{13 14}; their action can be enhanced if overdistension of the bladder and the accumulation of residual urine are avoided by regular complete voiding and by double micturition at least once daily.

Lenaghan *et al.*¹⁵ using intermittent short courses of antibacterial drugs for treating recurrent infections in 102 children with reflux, recorded a spontaneous cessation of reflux in only 42%. Altogether 66% of ureters of normal calibre and 26% of initially dilated ureters stopped refluxing over five to 18 years' observation compared with 85% of normal-calibre ureters and 41% of dilated ureters in our series. Moreover, Lenaghan *et al.* observed fresh scarring in 21% of initially normal kidneys, while we observed this in only 1%.

In each two-year period we found that reflux disappeared from 20-30% of the refluxing ureters being followed. This was probably an effect of maturation, the obliquity of the insertion of the ureter and hence its intramural length and valvular competence increasing with age.¹⁶ King *et al.*¹⁷ also regard the length of the intramural segment as an important guide to prognosis. This is also consistent with the smaller proportion of children in whom reflux stopped in the series of MacGregor and Freeman,¹⁸ where there was a mean follow-up of five years compared with seven to 15 years in this study (mean nine years).

The finding that reflux disappeared from 12 of the 19 scarred kidneys is consistent with Vermillion and Heale's observations¹⁹ in patients aged over 12 with chronic non-obstructive pyelonephritic scarring. They showed vesicoureteric reflux in only 45 out of 97 scarred kidneys, although 86 (89%) of the ureteric orifices were found on cystoscopy to have "abnormalities of such degree that reflux is likely to have occurred in the past," suggesting that childhood reflux had stopped. In view of these findings, the cystoscopic appearances are perhaps of less importance in determining management than the observation of normal renal growth without scarring.

Social, geographical, and temperamental factors in the child and the family must be considered in planning the long-term

management of each child with urinary tract infection and vesico-ureteric reflux, but any treatment programme should take into account the self-limiting nature of reflux in over three-quarters of the children affected.

This work has received generous financial support from the National Kidney Research Fund, Beecham Research Laboratories, the Medical Research Council, and University College Hospital research funds.

We are grateful for the help and co-operation of many medical and nursing colleagues, particularly Sister March, who has provided invaluable help in the clinic, and Mrs M Bedford for secretarial help.

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(Accepted 24 May 1977)

Release of copper from copper-bearing intrauterine contraceptive devices

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British Medical Journal, 1977, **2**, 288-291

Summary

We measured the rate of release from copper-bearing intrauterine contraceptive devices (IUDs) with varying copper surface areas. Over 135 days of observation the release rate decreased exponentially. This decrease in the

release rate was associated with the deposition of a protein layer on the surface of the metal. The bioavailability of copper on IUDs was not related to the volume of copper remaining on the device. Consequently any increase in the copper surface area of an IUD is unlikely to affect the duration of its copper-dependent contraceptive action.

Introduction

Early published data on the rate of copper loss from copper IUDs showed a uniform rate of release after an initial high loss¹ and have been used to calculate the duration of their use based on copper loss.² More recent studies have questioned the constant release-rate of copper and the long-term effectiveness of copper-bearing IUDs after finding that the copper acquires a coating of calcareous salts in utero that reduces its availability.^{3 4} The clinical importance of this observation has been vindicated

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by the reported increase in the rate of pregnancy associated with the use of copper-bearing IUDs after 24 months,⁵ and the report⁵ of lower pregnancy rate if the devices are replaced after two years. These findings suggest that the volume of copper remaining on an IUD may not be the most useful measure of its effective lifespan, and that increased copper loading may be of little value.⁶ Indeed the copper released into the uterus might instigate other biochemical events in the endometrium.

In this study we measured copper release from IUDs containing different surface areas of copper wire. We tried to simulate the in-vivo conditions as closely as possible by using an enriched medium with a protein content similar to that found in the uterine fluid,⁷ the release of copper into tissue culture being measured continuously.⁸

Materials and methods

The Copper Omega (A H Robins, Horsham) may have varying diameters of copper wire giving a wide range of copper surface areas. Devices containing 198, 252, 312, 391, 471, 555, 734, 868, 940, and 1112 mm² copper were used (the maximum deviation in copper surface area did not exceed 2% of the mean). Sterile technique was maintained throughout.

The devices were incubated in 15 ml of medium in an atmosphere of 5% CO₂: 95% air at 37°C. Fetal calf serum (Gibco-Biotech, Scotland) was included as it has a similar composition to human serum and is of highly standard composition. This also enabled us to compare results with the study of the effects of copper-releasing IUDs on cultured cells.⁹ Table I shows the constituents of the different media. For the long-term study of copper release medium I was used, and changed at 24-hour intervals.

TABLE I—Constituents of different media (I-IV) in which copper release was measured

Constituent	I	II	III	IV
Fetal calf serum (ml)	1.5	1.5		
Tissue culture medium (ml)	13.5		15.0	
(TC-199 + Hanks's salts + L-glutamine (pH 7.2))		13.5		15.0
Sterile distilled water (ml)				

The copper concentration was measured daily using temperature-programmed flameless atomic absorption spectroscopy with a lower limit of sensitivity of 78.7 pmol (5 ng) (Perkin Elmer heated graphite atomiser HGA-72 with a Varian Techtron AA-5 spectrophotometer). Molecular sieve chromatography was carried out with a 40 × 2.5 cm column of Sephadex G 200 equilibrated with the medium, the protein peaks being measured at 280 nm. The geometric and linear regressions were performed using a Wang 2200 mini-computer.¹⁰

Results

EFFECT OF PROTEIN ON COPPER RELEASE

We measured the release of copper from a device with 200 mm² copper surface area into either fetal calf serum, tissue culture medium, or both daily for seven days (table II). The release of copper into non-protein solutions was relatively constant after an initial high release during the first 24 hours. The highest release occurred in tissue culture medium alone and this was sustained throughout the seven days. A progressive decrease in copper release was seen in the presence of protein-containing solutions. The release of copper into aqueous fetal calf serum was 17 times lower than that into 10% fetal calf serum in tissue culture medium after seven days.

Since we thought the decrease in the release rate into protein-containing solutions was associated with the tarnished and blackened areas that developed on the surface of the copper we studied the effect of preincubating the IUD in 10% fetal calf serum in tissue culture medium for seven days (table III). When the IUD was transferred to pure tissue culture medium the rate of copper release was not significantly different from the release into tissue culture medium

TABLE II—Copper release from an IUD with 200 mm² copper surface area into various solutions measured daily for seven days. Variation in each measurement (n=4) is less than 10% of expressed mean

Medium	Daily release rate of copper (μmol)							Average daily release rate (μmol)
	1	2	3	4	5	6	7	
I Fetal calf serum + tissue culture medium	2.97	1.84	1.24	1.09	0.41	0.66	0.82	1.29
II Aqueous fetal calf serum	3.02	0.35	0.61	0.33	0.25	0.17	0.05	0.68
III Tissue culture medium	4.19	3.02	2.17	2.85	2.08	2.33	2.49	2.74
IV Water	0.130	0.042	0.014	0.019	0.017	0.011	0.008	0.020

Conversion: SI to traditional units—Copper 1 μmol ≈ 63.54 μg.

TABLE III—Copper release from IUD with 200 mm² surface area copper after preincubation for seven days in 10% fetal calf serum in tissue culture medium, when average daily release rate was 1.29 μmol (82 μg)

Medium	Average daily release (μmol)	Percentage decrease in copper release
10% fetal calf serum in tissue culture medium	0.55 ± 3%	57%
Tissue culture medium only	0.58 ± 4%	65%
Water	0.16 ± 1%	91%

Conversion: SI to traditional units—Copper 1 μmol ≈ 63.54 μg.

containing fetal calf serum. This suggests that the layer acquired prevented the high rate of sustained release previously observed in tissue culture medium alone (table II).

We examined the fate of copper on release into solution by molecular sieve chromatography of the complete incubation medium on Sephadex G 200 (fig 1). The copper in solution existed in two forms.

FIG 1—Elution profile from Sephadex G-200 of copper (●) and protein (▲) present in tissue culture medium containing 10% fetal calf serum after 135 days' continuous contact with a copper-bearing IUD.

One was associated with a protein peak which had an elution characteristic identical with pure albumin. The second copper peak occurred in the salt peak—that is, with the low molecular-weight components.

Exchange of copper between the two forms in solution occurred freely as shown by the dialysis of the "albumin" peak against fresh copper-free tissue culture medium. The copper diffused freely until a new equilibrium was established. Thus the protein-bound copper in solution was in a free equilibrium with the low molecular-weight copper fraction.

COPPER RELEASE FROM DIFFERENT SIZES OF IUD

Copper-bearing IUDs with surface areas of 198-1112 mm² copper were incubated for 135 days in 10% fetal calf serum in tissue culture medium, the medium being changed at 24-hour intervals. In all cases the copper release decreased exponentially during the study (fig 2). The most rapid drop in copper loss occurred during the first 30 days, though the release rate continued to decrease throughout the 135 days.

The average release rates were calculated for five intervals during the study—namely, days 1-7, 8-15, 16-30, 31-60, and 61-135 (table IV). The rates of release during these periods all approximated to straight lines which were derived by linear regression (correlation coefficients 0.97-0.82; see table V). This shows that the release rate was directly proportional to the copper surface area during the 135 days. Nevertheless, the dependence of the copper release rate on the copper surface area did fall with time as shown by the change in the slopes of the lines in fig 3. The slopes of these lines are equal to the numerical value of the x-term in the regression equation (table V), and define the relation between the copper surface area on the IUD and the rate of copper release. When these figures are compared with the duration of use of the IUD they describe an exponential function which was found by geometric regression (correlation coefficient 0.983). This equation shows an exponential decline in the dependence of the rate of copper loss on the surface area of copper as the time of

FIG 2—Daily release of copper from an IUD with 198 mm² copper surface area showing standard deviation.

TABLE IV—Total copper release (μmol) over 135 days from IUDs with different copper surface areas. Daily averages are given in parentheses. Variation for each measurement never exceeds 10% of mean

Copper surface area (mm ²)	Days of study				
	1-7	8-15	16-30	31-60	61-135
198	8.99 (1.275)	3.15 (0.582)	6.63 (0.441)	10.47 (0.362)	10.06 (0.135)
252	11.93 (1.7)	4.12 (0.519)	6.04 (0.409)	10.95 (0.362)	11.57 (0.156)
312	14.05 (2.014)	4.33 (0.614)	8.97 (0.598)	11.38 (0.378)	10.59 (0.143)
391	18.95 (2.707)	11.51 (1.432)	9.77 (0.645)	11.73 (0.378)	11.90 (0.161)
471	22.51 (3.211)	8.69 (1.023)	8.72 (0.582)	15.42 (0.519)	28.69 (0.387)
555	22.55 (3.226)	7.57 (0.944)	7.90 (0.535)	13.93 (0.472)	29.75 (0.401)
734	26.63 (3.809)	9.84 (1.228)	8.64 (0.567)	17.85 (0.614)	23.99 (0.324)
868	39.53 (5.65)	27.98 (3.856)	16.08 (1.07)	23.95 (0.803)	25.87 (0.346)
940	36.12 (5.146)	21.55 (3.085)	21.94 (1.464)	23.54 (0.787)	29.05 (0.393)
1112	39.83 (5.681)	25.37 (3.62)	29.04 (1.936)	41.17 (1.369)	57.90 (0.774)

Conversion: SI to traditional units—Copper 1 μmol ≈ 63.54 μg.

TABLE V—Regression equations used to calculate average rate of copper release from IUDs of all sizes (198-1112 mm² copper surface areas) during five intervals of study period (see fig 3)

Interval (days)	Regression equation	Correlation coefficient
1-7	0.3166406x + 34.0035126	0.97
8-15	0.238947x + 37.0777622	0.89
16-30	0.0900845x + 0.236266	0.82
31-60	0.060272x + 4.5433226	0.91
61-135	0.0336648x + 0.8833058	0.85

FIG 3—Release rate of copper from all sizes of copper IUD at five intervals during study period. Lines fitted by linear regression (see table V).

FIG 4—Graphical expression of decrease in dependence of copper release upon copper surface area of the IUDs (expressed as slope of lines in fig 3) as a function of time.

FIG 5—Probable route of uptake of copper from IUDs in vivo, showing copper-amino-acid-albumin surface complex.

use increases (fig 4). The equation suggests that the release of copper from IUDs continues to fall throughout their period of use. The rate of copper loss becomes less dependent on the copper surface area, as the surface layer which forms on the copper probably inhibits continued copper release. This effect cannot be avoided by increasing the copper content of the IUD.

Discussion

Our figures for the rates of copper release from IUDs during the first month of use comply with the in-vivo figure of 0.71 μmol/day (45 μg/day) obtained elsewhere for a device with 200 mm² copper surface area.⁶ There is some disagreement in the published data on the change in the rate of copper release during

the use of the device. Hagenfeldt¹ reported a constant rate after the first 60 days, while Timonen⁶ found a steady decline in the rate of loss. The regression equation we derived showed a decline in the rate of copper loss from all sizes of device throughout their use, which gradually became less dependent on the available copper surface area. If this equation is valid for longer periods of time we predict that a 200-mm² copper IUD would lose 0.046 μ mol/day (2.9 μ g/day) copper after one year of use, 0.027 μ mol/day (1.7 μ g/day) after two years, and 0.019 μ mol/day (1.2 μ g/day) after three years. Thus a device with 5400 mm² copper surface area would be required to obtain the reported release rate of 0.71 μ mol/day (45 μ g/day) after two years. These figures suggest that the long-term use of IUDs dependent on copper for their contraceptive action is of dubious value. Furthermore, a recent report³ showed a significant increase in the failure rate of copper IUDs after two years' use, which could be prevented by replacing the device at that time.

The decrease in available copper was probably related to the appearance of the surface layer on the copper. In this short study we identified the early deposit as a protein layer, though long-term in-vivo studies show a calcareous deposit formed after extended use.³ We could not determine the chemical composition of the protein layer, though albumin was probably one of the active constituents.

Investigations suggest that the presence of a copper-albumin surface complex on IUDs is important in the facilitation of copper removal.⁷⁻¹¹ We found that the low molecular-weight components of tissue culture medium were important in sustaining the release of copper, and that copper loss was inhibited by protein-containing solutions. The presence of albumin in the surrounding medium contributes to the copper release, as an equilibrium exists between the copper associated with the low molecular-weight components of the tissue culture medium

and that which is bound to the albumin in solution. A similar dual location for the free copper probably exists in vivo, where a copper-amino-acid-albumin complex has been suggested as an intermediate step in the uptake of copper (fig 5).¹² The observed uptake of copper from IUDs probably occurs by this route, with the subsequent rapid excretion of this excess copper.¹³

We are indebted to Miss K Lo, Mrs T Puri, and Mr P Scudder for their technical help; and to Dr Charles Emmett, inventor of the Omega device, for arranging for the range of IUDs used in this study to be made.

EC was supported during this study by a research fellowship granted by A H Robins, Horsham.

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(Accepted 24 May 1977)

Acetylator phenotype in diabetic neuropathy

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British Medical Journal, 1977, **2**, 291-293

Summary

The proportions of slow and fast acetylators in a group of diabetics with symptomatic peripheral neuropathy were compared with those in a group of diabetics who had had the disease for at least 10 years without developing neuropathy. There was a significantly higher proportion of fast acetylators in the group of diabetics without neuropathy than in those with neuropathy or in the normal population.

Hence genetic factors separate from the diabetic

diathesis may determine the development of neuropathy in any particular diabetic.

Introduction

The exact pathogenesis of the complications of diabetes is unknown but they are thought to be secondary to the metabolic abnormality, and they increase with the duration of the disease and poor control.¹ Only a few patients, however, develop complications regardless of the duration of their diabetes. Thus the overall prevalence of symptomatic peripheral neuropathy is about 20%,² yet in a group who had had diabetes for more than 40 years the prevalence of neuropathy was only 15%.³ Why only this small proportion of patients develop neuropathy in the presence of a presumably common metabolic abnormality is unknown, but possibly there is a genetic susceptibility to complications.

The rate of acetylation of certain drugs, including the anti-tuberculosis drug isoniazid and sulphonamides, is genetically determined, and the population may be divided into slow and fast acetylators.⁴ Slow acetylators are more likely to develop neuropathy when treated with isoniazid.⁵ Because a similar genetic dimorphism might influence the development of neuropathy in diabetes we decided to determine the acetylator phenotypes of diabetics with and without neuropathy.

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