serum level and the previous peritoneal findings. That lysozyme levels may in fact be raised in renal failure patients is known (Prockop and Davidson, 1964), because in uraemic patients there is diminished catabolism of lysozyme by the kidney (Hansen *et al.*, 1972). The obvious limitation is that large doses of heparin might inhibit the assay. The kinetics of such inhibition have been described by Kerby and Eadie (1953). As has been shown, however, the amount of heparin normally used in our dialysis scheme will not affect the results.

Lysozyme, an enzyme of M.W. 15,000, is normally present in tears, external secretions, and white cells (Osserman and Lawlor, 1966). It is absent from lymphocytes (Barnes, 1940) but is present in monocytes and macrophages (Myrvik *et al.*, 1961). That it is also derived from granulocytes seems likely since the highest serum levels are found in monocytic and myelomonocytic leukaemia, and there is some elevation in all myelogenous leukaemia and during the course of bacterial infections (Crowder and White, 1968). Probably the lysozyme present in normal peritoneal fluid is partly derived from the serum and partly from white cells resident around the peritoneum. Hence in the presence of peritoneal infection a rise is to be expected and, with increasing permeability of the peritoneal membrane, levels greater than those of serum might be expected. Lysozyme is released from white cells by endotoxin or in inflammatory reactions (Tew *et al.*, 1971).

Turbidity of cooled peritoneal fluid due to a high protein content seems to occur during the course of dialysis without necessarily implying infection, although it must indicate serous exudation. That this is so is indicated by the patients in the table listed as having a score of unity. This suspicion is further reinforced by the observation that for some reason turbidity can be intermittent. Also on one occasion a positive culture was obtained as a result of contamination, for there was no other indication of infection. Hence an additional way of diagnosing peritoneal infection is needed. Chronic renal failure patients

are susceptible to infection and Stewart *et al.* (1966) recorded peritonitis in 6% of all treatments, and a positive blood culture appeared in another 12% during the course of four-day dialyses.

This study indicates that the use of lysozyme assay will not only aid patient management but also be of value for studies of peritoneal infection.

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PRELIMINARY COMMUNICATIONS

Effect of Copper-containing Intrauterine Contraceptive Devices on Human Cells in Culture

R. W. JONES, N. M. GREGSON, MAX ELSTEIN

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Summary

Intrauterine devices containing copper were introduced into cultures of adult and fetal cells. The Dalkon shield had no apparent effect on cell growth. The Gravigard device caused inhibition of growth and an increase in

copper concentration in the supernatant medium. When the latter device was transferred through a series of subcultures the rate of release of copper decreased and growth was no longer inhibited.

Introduction

The addition of copper wire to the surface of an inert intrauterine device (I.U.D.) has been the most significant advance in recent years (Zipper *et al.*, 1969; Newton *et al.*, 1972). Copper and its salts have also been introduced into the plastic matrix of at least one new device (Davis, 1971). The evidence at present suggests that the site of action of the copper is confined to the uterus itself (Zipper *et al.*, 1969; Chang and Tatum, 1970) though this may not be the only mode of action (Elstein and Ferrer, 1970). Nevertheless, concern has been expressed regarding the use of pharmacologically active and potentially toxic materials in I.U.D.s (Wolters, 1972). The possibility of inducing teratogenic effects in man cannot necessarily be excluded by experiment in animals whose reproductive physiology often differs greatly from that of man.

It was therefore decided to examine the effects of two new, though quite different, copper-containing I.U.D.s on human and fetal tissue culture systems. In one device (Dalkon shield, A. H. Robins) the copper is largely within the plastic matrix and in the other (Gravigard, G. D. Searle) a copper wire is wound on to the inert plastic stem of the device.

Department of Human Reproduction and Obstetrics, Southampton University Hospital, Southampton

R. W. JONES, F.R.C.S., M.R.C.O.G., Lecturer
 MAX ELSTEIN, M.D., M.R.C.O.G., Senior Lecturer

Cytogenetics Unit, Department of Pathology, Salisbury General Hospital, Salisbury
 N. M. GREGSON, PH.D., Cytogeneticist

Materials

Intrauterine Devices.—The devices used in the study were: (a) the Dalkon shield consisting of a semipermeable plastic (ethylene vinyl acetate) with barium sulphate (12%), copper dust (0.5%), and anhydrous cupric sulphate (1%) shaped to conform to an “average” uterine cavity. There is a thin central membrane to increase endometrial contact. (b) The Gravigard (Copper 7) made of a polypropylene and polyethylene copolymer, with added barium sulphate, shaped like a figure 7. Copper wire (0.203 mm diameter) is wound round the vertical limb to give a 200 mm² surface area of copper. (c) Devices identical to (a) and (b) but without copper (for use as controls).

Tissue Cultures.—The tissue cultures used were: amniotic fluid cells in a range of gestational ages from 14 to 32 weeks obtained at hysterotomy or by amniocentesis; and monolayers of fibroblasts and epithelioid cells obtained by culture of fetal skin, adult skin, and amniotic fluid cells.

Method

Leighton tubes containing cover slips and 1 ml of TC 199 enriched with 25% fetal calf serum were seeded with cells and incubated at 36.5°C. Standard portions of I.U.D. were placed in some tubes while duplicates were maintained as controls.

Amniotic Fluid Cells.—These were maintained using the method of Gregson (1970) and harvested for chromosome analysis when two or three colonies of confluent cells were observed.

Monolayer Cultures.—The copper-containing devices were passed through a series of subcultures. Twenty-four hours after each subculture the state of growth was recorded; the supernatant medium was sampled for estimation of its copper content by the Boehringer kit method (Zak, 1958); and cover slips were removed for detailed examination and photography of the cells, trypan blue staining to determine the proportions of live cells in the culture (Girardi *et al.*, 1956), and histochemical staining of the cells with rubanic acid for detection of copper (Uzman, 1956). Cultures having a range of different concentrations of copper in their supernatant media were harvested for chromosome analysis.

Results

AMNIOTIC FLUID CULTURES

The results from analysis of variance showed that the growth rate of cultures containing Dalkon shields impregnated with copper was not significantly different from that of the controls (Table I). Growth was not observed in cultures containing Gravigards (table I).

TABLE I—Results from Cultures of Amniotic Fluid Cells

Intrauterine Device	No. of Cultures Established	No. of Days between Seeding and Harvesting	
		Mean	Range
No device	25	12	8 to 17
Dalkon shield without copper	14	13	8 to 17
Dalkon shield impregnated with copper	22	14	10 to 24
Gravigard without copper wire	5	13	8 to 15
Gravigard with copper wire	11	—	—

MONOLAYER CULTURES

The monolayer cultures derived from adult skin, fetal skin, and amniotic fluid cells all showed similar results and will be considered together.

The eight cultures containing Dalkon shields impregnated with copper all showed normal healthy monolayers 24 hours

after seeding. The monolayers continued to grow normally and the copper content of the medium never exceeded 18 µg/ml, being in the same range as the controls. No copper was detected in the cells.

Observations were made on 16 cultures containing Gravigard devices. Serial estimations of the copper content of the supernatant media were recorded from nine of them with their nine controls. Table II gives the results of a regression analysis (grouped data) of copper concentration in µg/ml (x) against the log of the number of occasions of use (y). There was a significant

TABLE II—Regression Analysis (Grouped Data) Copper Concentration (µg/ml) / Log. Occasions of Use (Bishop, 1971)

	S.S.	D.F.	M.S.S.	V.R.	
Regression	419702	1	419702	66.4	P<0.1%
Deviation from regression	37926	6	6321		
Total between occasions of use	457629	7	65376	8.5	P<0.1%
Total within occasions of use	245495	32	7672		
Total variation in “y”	703124	39			

S.S. = Sum of squares. D.F. = Degrees of freedom. M.S.S. = Mean sum of squares. V.R. = Variance ratio.

relation between the two parameters which could be described by the equation $y = 294.79 - 361.58x$ ($b = 361.58 \pm 44.37$). The copper concentration in the supernatant medium declined significantly as the number of occasions of use of the Gravigard increased (see fig. 1). Throughout the experiments there was agreement between the copper concentrations of the media and

FIG. 1—Relation between copper concentration and log. of number of times device used.

the observed state of growth (table III). Trypan blue staining showed that a high proportion of the rounded off cells were dead. Large amounts of copper were present in these cells as shown by the dense staining with rubanic acid (fig. 4). The controls remained unstained. Comparable results were obtained when cells were cultured in medium containing copper chloride in equivalent concentrations to those eluted from the I.U.D.s.

TABLE III—Results from Monolayer Cultures

Copper Concentration in Media	Observations on Cell Growth
Below 50 µg/ml	Normal healthy monolayer (fig. 2)
50 to 150 µg/ml	Variable proportions of healthy to rounded off cells. Round cells found particularly in those regions which were in close contact with the Gravigard
Above 150 µg/ml	Most attached cells were rounded off (fig. 3)

FIG. 2—Normal healthy monolayer of fibroblasts. (Phase contrast microscopy. $\times 95$.) FIG. 3—Rounded off fibroblasts from culture with copper concentration in supranatant medium of 414 µg/ml. (Phase contrast microscopy. $\times 95$.)

Eight cultures were harvested for chromosome analysis. No cells were observed in metaphase from those with high concentrations of copper in the supranatant medium. Seventeen metaphases were counted from the three cultures with concentrations of copper below 50 µg/ml. No chromosome abnormalities were detected but complete analysis was possible on only five cells.

Discussion

The above experiments offered the opportunity for studying the effects of copper on rapidly dividing fetal and adult cells in vitro but lacked the dynamic processes which occur in vivo. The Dalkon shield had no discernible effect on any of the cultures, suggesting that the copper plays no active contraceptive role here. It is now apparent that the sole function of the copper is to

FIG. 4—Fibroblast monolayer stained with rubeanic acid and showing large deposits of copper in all cells (copper concentration of supranatant medium = 192 µg/ml). (Light field microscopy. $\times 95$.)

strengthen and at the same time improve the moulding properties of the plastic (I. Lerner, personal communication, 1972). Microscopical examination indicates that only a minute proportion of copper is on the surface of the shield and dissociation studies indicated that less than 5% of the total copper is released (Robins, 1972). Our data on copper levels in the supranatant media supports this.

The Gravigard device had a noticeable initial effect on the tissue cultures. In these static in-vitro cultures the cells initially changed their physical characteristics, failed to divide, and soon died. Nevertheless, passage of the same Gravigard through a series of subcultures in time allowed the cells and cultures to retain their physical characteristics and divide. Oxidative changes are noted on the surface of the copper wire after use, suggesting that this process influences the release of copper ions. Hagenfeld (1972) found a much higher release of copper in women during the first two cycles of use and after this a relatively constant release for one year. Our data confirm the higher initial release of copper. This may be of significance, providing greater effectiveness at a time when inert I.U.D.s are known to have their maximum pregnancy rates (Tietze and Lewit, 1970).

Early in vitro experiments suggested that copper is toxic to animal embryos in low concentration (Whitten, 1971). Whittingham (1972) showed that the I.U.D. containing copper wire does not have a direct toxic effect on the blastocyst since the protein in uterine secretion may provide protection from the free copper ions. The present study supports this latter view, though a direct toxic action cannot be excluded in the interval immediately after insertion.

While there is evidence of some systemic absorption of copper in rats (Okereke *et al.*, 1972) there is to date no suggestion that this occurs in man (Hagenfeld, 1972). The exact relation, locally, between free and bound elemental copper in the proteins remains obscure. No apparent abnormalities have been reported in laboratory animals developing in a copper milieu, and the few babies delivered after pregnancies with copper wire I.U.D.s in situ have apparently been morphologically normal (J. Zipper, personal communication, 1972). This does not, however, exclude subtle biochemical changes, and until long-term studies are completed this question must remain open. The lack of chromosome abnormalities in the human and fetal cells observed to date are, however, reassuring.

In conclusion we believe that the Dalkon shield is inert and has no biological action. In this experiment, however, the Gravigard device produced noticeable initial effects on adult and fetal tissue cultures. In the long term these effects gradually disappeared. Hence probably, in the dynamic situation, this device may not be harmful to such tissues.

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Requests for reprints should be addressed to: Dr. N. M. Gregson, Cytogenetics Unit, Department of Pathology, Salisbury General Hospital, Salisbury, Wiltshire.

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Thermography in Diagnosis of Deep Venous Thrombosis

E. D. COOKE, M. F. PILCHER

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Summary

The possibility that subclinical increased heat of the limb may be an early and reliable sign in the diagnosis of deep venous thrombosis is being investigated using thermography. Of the 35 cases studied 17 have had early deep venous thrombosis confirmed on phlebography. Of these, 16 cases have been previously diagnosed using thermography.

Introduction

Clinical signs of deep venous thrombosis occurring in the legs are unreliable and often absent (Pilcher, 1939; Greaves, 1940; Cosgriff, 1947; Coon and Willis, 1959; Sevit and Gallagher, 1959; Uhland and Goldberg, 1964). The first evidence that such thrombosis has occurred may be fatal pulmonary embolism (Coon and Coller, 1959; Sevit, 1962). Lambie *et al.* (1970), studying 111 surgical patients considered to be at high risk, found that almost half of the patients developed deep venous thrombosis. Of these cases two-thirds were unsuspected clinically despite careful scrutiny, and of those diagnosed clinically the diagnosis was falsely positive in a quarter. Kemble (1971), emphasizing the latter point in a report on a similar study of 280 patients, found that in those in whom a clinical diagnosis was made, in almost 40%, when subjected to further investigation, no thrombus was found.

It appears certain that in a susceptible patient deep venous thrombosis can occur early during enforced recumbency, whether this be postoperative or due to a medical condition such as myocardial infarction, or even during an operation (Flanc *et al.*, 1968; Negus *et al.*, 1968; Negus *et al.*, 1969). There may be no clinical evidence, save perhaps the development of pulmonary embolism. Negus (1967) showed that in cases of massive fatal pulmonary embolism fewer than 25% are preceded by warning signs in the legs or chest.

In a detailed postmortem study, Morrell and Dunnill (1968) indicated that in the United Oxford Hospitals between July 1964

and July 1965 there were 148 unnecessary and therefore, at least in theory, preventable deaths from pulmonary embolism in the hospital population. Owing to circumstances their study included a low incidence of orthopaedic cases. If it had not the figure could well have been higher.

Clearly, then, the early diagnosis of deep venous thrombosis and identification of the patient at risk is mandatory if mortality is to be reduced and the morbidity of the postphlebotic syndrome is to be avoided. This can be obtained only by a constant awareness of the condition and special investigation, otherwise we will observe, with Pilcher (1939), that "in the majority of non-fatal embolisms no evidence of thrombosis was found. The majority of fatal embolisms were unexpected."

Accepting, then, that clinical signs of deep venous thrombosis of the legs were frequently absent or misleading, that a clinically normal leg could be the site of a life-endangering, propagating thrombus, it appeared to us equally evident that a method of special investigation applicable to all cases and which was non-invasive, and therefore without risk to the patient, was an urgent necessity. Easy and rapid application and ready repeatability were other factors considered.

On reviewing the literature and from personal experience it appeared to one of us (E.D.C.) that the most constant clinical sign, present when other signs were either absent or equivocal, was an increase in the heat of the limb and delayed cooling on exposure, a feature emphasized by Pilcher (1939). Consideration of the chemical activity in the limb accompanying and consequent on a thrombotic episode suggests the possibility that increased heat could be present but subclinical from an early stage. Thus a means of measuring this increased heat would possibly present a method by which deep venous thrombosis could be detected early in its development. The developments in thermography offer such a technique.

Thermographic System

Thermography shows the temperature difference on an object in the form of a thermal picture (grey-tone thermogram) utilizing the natural infrared radiation, which varies with the surface temperature of the object. The thermographic system used in this study was the AGA Thermovision system 680, which operates in a similar fashion to a closed-circuit television, in which a camera is coupled direct to a display unit by an interconnecting cable.

The camera unit consists basically of a single-element indium antimonide detector cooled with liquid nitrogen to attain high sensitivity in the spectral band 2.5-6 microns. A unique refractive optical system mechanically scans the object and brings the infrared rays to a focus on the detector. This scanning principle produces a real-time thermal picture (16 fields/sec) with good thermal resolution—that is, better than 0.2°C at 30°C—and a spatial resolution of 1.3 milliradians of instantaneous field of view with the 10° lens as used in this study. The video signal from the

Hackney Hospital, London E9 6BE

E. D. COOKE, M.B., B.CH., Clinical Assistant

M. F. PILCHER, F.R.C.S., Consultant Orthopaedic Surgeon