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COMMUNICATIONS

The determination of leachable chromium from chromicized catgut sutures

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Application of the British Pharmacopoeia (BP) Analytical procedure for the determination of leachable chromium to samples of catgut sutures yielded fading coloured solutions and was considered to constitute a poor basis for an analytical method. The United States Pharmacopeia (USP) method and an adaptation of the American Public Health Association (APHA) method were explored and found to give linear responses over the concentration range of interest. Both these methods were applied to a series of samples and the results obtained were different as the APHA method measures total chromium while the USP procedure determines only chromium (VI).

The monographs of the British Pharmacopoeia (BP) constitute the official requirements for sutures in Australia although the majority of sutures available are manufactured to the specifications of the United States Pharmacopeia (USP). The treatment of catgut sutures with chromium compounds to prolong the strength (Postlethwaite 1975; Sugimachi et al 1978) of the sutures in-vivo has the disadvantage that chromium salts may leach from the treated sutures into the healing wounds. Both the USP and BP monographs include semi-quantitative tests which utilize the reaction between chromium VI and diphenylcarbazide to determine levels of leachable chromium in chromicized catgut sutures. The BP method involves an oxidation step and provides a result for total chromium whereas the USP procedure determines the level of chromium (VI). A limit of 100 μg of chromium per gram of suture is specified by both Pharmacopoeias.

The work reported is part of a survey undertaken to assess the relevance of the official requirements to the quality of the sutures on the market. The study included testing the physical performance (Lee et al 1983) and chemical attributes of the sutures. Application of the BP analytical procedure to samples of catgut sutures yielded an orange solution that faded in intensity and included a precipitate while a stable red-violet colour was obtained with the standard solutions. It was considered that the formation of different colours for the sample and standard solutions constituted a poor basis for an analytical method and alternative procedures for the determination of chromium in catgut sutures were investigated.

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Materials and methods

Sample preparation. A sample of about 250 mg suture accurately weighed was placed in a conical flask containing 25 ml of water, stoppered and allowed to stand at 37 °C for 24 h. The supernatant was decanted and the content of chromium determined by comparison with a series of dilutions of the standard solution of potassium dichromate (28·4 μg $K_2Cr_2O_4$ ml^{-1}).

Assay procedures

American Public Health Association (APHA). The following colorimetric procedure is essentially that published by the American Public Health Association (APHA) for the determination of chromium in waste water (1980) with the sections relating to the treatment of samples and the separation of molybdenum, vanadium, iron and copper with cupferron deleted.

A 10 ml volume of sample solution was pipetted into a 125 ml conical flask and 1 ml of 50% v/v sulphuric acid added. The volume was adjusted to approximately 30 ml with distilled water, a boiling chip added and the solution heated until boiling. Two drops of 4% w/v potassium permanganate was added and the dark red colour of the solution was maintained through the dropwise addition of the permanganate solution. After 2 min, 1 ml sodium azide solution (0·5% w/v) was added. In those instances where the red colour did not fade completely within 30 s an additional 1 ml volume of sodium azide solution was added. The solution was then boiled for a further minute, cooled and 0·25 ml phosphoric acid was added.

The cooled solution was transferred to a 50 ml volumetric flask, 2·0 ml diphenylcarbazide solution added and made up to volume with distilled water. The solution was allowed to stand for 10 min before determining the absorbance at 540 nm in a 1 cm cell. A 10 ml sample of distilled water treated in the same manner as the sample was used as a reagent blank.

The concentration of chromium present in the sample was determined by comparison with a standard curve based on the absorbance values of 1, 2, 3, 4, 5, 6, 8 and 10 ml volumes of the standard dichromate solution treated in the same manner. (10–100 μg Chromium added = 0·2 to 2 μg chromium in final solution.)

USP XX. The determination of leachable chromium was performed in accordance with the procedure specified in the USP XX monograph for Absorbable Surgical Sutures.

BP 1980. The determination was performed in accordance with the procedure specified in BP 80 monograph for Sterile Catgut Sutures. As the sample preparation contained an oxidation step, the effect of including the oxidation step in the treatment of the standard solutions was investigated.

Results and discussion

The absorbance values obtained for standard potassium dichromate solutions using the USP XX and APHA methods are provided in Table 1. The results obtained in both instances exhibit good linearity (Fig. 1) and reproducibility. The two procedures were applied to the determination of leachable chromium for commercial chromicized catgut sutures and the results obtained are shown in Table 2. For samples 1, 3, 4 and 7 the levels of total chromium as determined by the APHA method were 5 to 30 times the level of chromium VI obtained by the USP procedure. It is possible that these high results could be due to 'catgut dust', produced in polishing adhering to the surface and crevices of the suture and being dislodged by steeping in water at 37 °C. The collagen/chromium III complex would be hydrolysed and oxidized to chromium VI by the subsequent treatment.

Table 1. Determination of chromium levels - linearity studies.

Standard Concn (μg Cr)	Absorbance BP	USP	APHA
2·04	0·255	0·115	0·018
4·1	0·33	0·235	0·039
6·1	0·26	0·348	0·064
8·2	0·3	0·462	0·085
10·2	0·25	0·572	0·108
20·4	0·33	—	0·22
30·4	0·25	—	0·338
40·0	0·3	—	0·445

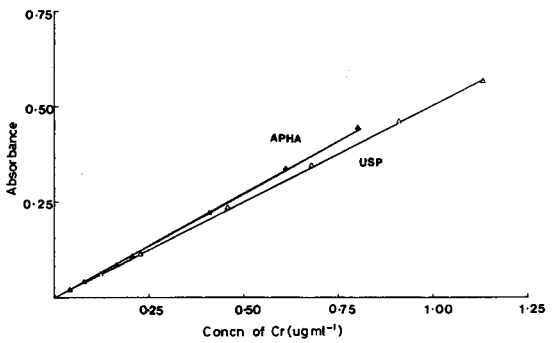

FIG. 1. Linearity studies of the USP and APHA procedures.

The procedure specified in the BP 80 includes a persulphate oxidation step in the treatment of the sample but not the standard solutions. In our hands application of the BP procedure to solutions obtained from samples yielded a yellow-orange solution containing a precipitate while a red-violet solution was obtained for the standard solutions. The absorbance values obtained for a range of standard solutions using the BP procedure (persulphate oxidation step included) were unrelated to the concentration of chromium (Table 1) and illustrate the difficulties encountered by this laboratory in applying the BP procedure.

Table 2. Leachable chromium from chromicized catgut sutures.

Sample	USP* (μg g^{-1})	APHA (μg g^{-1})
1	75	1540, 1280, 1330
2	4	0
3	44	250, 220, 216
4	2	28
5	1	0
6	4	2
7	1	31
8	<1	1

* The USP specification is not more than 1 μg ml^{-1} of solution or 100 μg g^{-1} suture.

The USP XX procedure determines the level of chromium (VI) while the BP 80 and APHA procedures determine the level of total chromium leached from the suture under the test conditions. In each instance the limit is prescribed as not more than 100 μg g^{-1} suture. Chromium (VI) is a strong oxidant and the USP XX limit would appear to be based on the premise that chromium (VI) is more likely to cause tissue irritation than other chromium species. On the basis of the results obtained by this Laboratory the procedure developed by the American Public Health Association is preferred to the BP 80 procedure for the determination of soluble chromium compounds. It is suggested the limit of 100 μg chromium per gram of suture presently specified in the BP 80 monograph should be retained as the restriction on the total amount of leachable chromium will ensure the sutures are well rinsed during the manufacturing process.

Conclusion. The results obtained through application of the analytical procedure for leachable chromium specified in the British Pharmacopoeia monograph for catgut sutures exhibit poor reproducibility and linearity. The United States Pharmacopeia method for the

determination of leachable chromium VI in sutures provides reproducible results with acceptable linearity. The American Public Health Association procedure has the advantage of determining precisely the total amount of leachable chromium present in the sutures and it is recommended this procedure be adopted together with the BP limit in the official requirements for these products.

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Warfarin—sulfinpyrazone interaction on binding to human serum albumin

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Sulfinpyrazone displacement of warfarin from human serum albumin was studied in-vitro. At low sulfinpyrazone concentrations one molecule of warfarin is displaced on binding by one molecule of sulfinpyrazone. Clinical plasma concentrations of sulfinpyrazone are, however, too low to cause significant displacement.

Haemorrhage during combined treatment with warfarin and sulfinpyrazone has repeatedly been reported (Weiss 1979; Bailey & Reddy 1980; Gallus & Birkett 1980). The mechanism involved is probably reduced metabolic clearance of *S*-warfarin (O'Reilly 1982). A possible role of competition of the two drugs for binding to plasma albumin has been suggested by Bailey & Reddy (1980) but this effect has not been studied in-vitro although Seiler & Duckert (1968) have demonstrated that sulfinpyrazone displaces another coumarin anticoagulant, phenprocoumon, from binding to albumin.

Materials and methods

Human serum albumin was obtained from AB Kabi, Stockholm, Sweden (Lot Nr. 75953) and was defatted with charcoal in acid solution, lyophilized and stored at 4 °C (Chen 1967). [^{14}C]Warfarin (3-α-acetonyl[α-^{14}C]-benzyl-4-hydroxycoumarin) was obtained from The Radiochemical Centre (Amersham, UK) and purified by tlc using toluene–dioxane 9:1 and tested. The specific activity was 177 μCi mg^{-1}. Sulfinpyrazone was a gift from Ciba-Geigy A/S (Copenhagen). The interaction between warfarin and sulfinpyrazone was investigated by utilizing a recently developed technique for dialysis rate determination (Brodersen et al 1982). Twenty μl of a solution of [^{14}C]warfarin and albumin in buffer was placed on one side of a cellophane membrane with 20 μl of an identical buffered albumin solution on the other side. Dialysis was for 20 min at 37 ± 0·3 °C. Sulfinpyrazone was added in varying amounts, equally on both sides of the membrane. An increased dialysis rate is observed if warfarin is displaced by the second drug. [^{14}C]Warfarin concentration on either side of the membrane was measured by liquid scintillation counting.

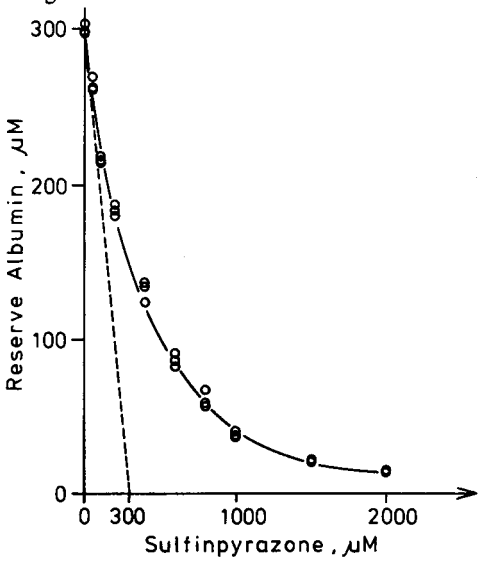

FIG. 1. Reserve albumin-equivalent for binding of warfarin as a function of sulfinpyrazone concentration, measured by rate of dialysis in a 300 μM solution of defatted human serum albumin in 66 μM sodium phosphate buffer, pH 7·4, 37 °C.

Results and discussion

The reserve albumin-equivalent for binding of warfarin in a sample containing sulfinpyrazone is defined as the concentration of albumin in pure solution which will bind warfarin equally tight as in the sample (Brodersen et al 1982). Results are seen in Fig. 1 where the reserve albumin-equivalent for binding of warfarin in a 300 μM (20 g litre^{-1}) human serum albumin solution containing 10 μM of racemic warfarin is plotted on the ordinate with varying concentrations of sulfinpyrazone on the

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