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Technical method

Automated method of nitrite estimation in gastric juice

PCH WATT, JOAN D DONALDSON *From the Department of Surgery, Queen's University of Belfast, Belfast*

The suspected link between nitrite and gastric cancer has led to several reports on the relation between pH and nitrite concentration in human gastric juice.[1–3] Several methods have been used to estimate nitrite in biological fluid. Chemiluminescent[4] and polarographic[3] techniques both require complex equipment. The commonest methods described are based on the colour reaction of Greiss. These require prior protein precipitation, which may interfere with results.[5] In addition, there is a possibility that bilirubin or biliverdin might interfere with the colour reaction.

Laboratories concerned with the investigation of nitrite in gastric juice are often presented with many samples for analysis. This is because the nitrite concentration in gastric juice is so variable that many samples are taken from one patient to assess 24 h exposure.[3] Also single samples may be taken from large populations in epidemiological studies.[6] A manual method in these circumstances is a disadvantage.

We report here the use of the Chemlab nitrate/nitrite cartridge for rapid automated analysis of nitrite in human gastric juice in an attempt to overcome some of the problems of previous techniques.

Material and methods

A Chemlab nitrate/nitrite cartridge was used in conjunction with a Chemlab automatic continuous flow system. Only the nitrite part of the apparatus was used. The procedure used was that described by Chemlab (Chemlab Instruments, Hornchurch, Essex) (Method sheet no CW2-066-01) for analysis of nitrite in water. Since the cartridge contains a dialyser membrane, which allows only compounds less than 500 daltons through, no prior protein precipitation was required. The effectiveness of the dialyser membrane in removing bilirubin was assessed as many gastric juice samples are contaminated by bile.

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Gastric juice was collected from 85 subjects as described elsewhere (p 511).

The pH of samples was measured using a Corning pH meter model 113. Samples were centrifuged at 3000 rpm at 4°C for 15 min after being saturated with borax, and the supernatant was stored at −20°C (to prevent nitrite breakdown at low pH) until sufficient was available for the autoanalyser. Approximately 1 ml samples were required.

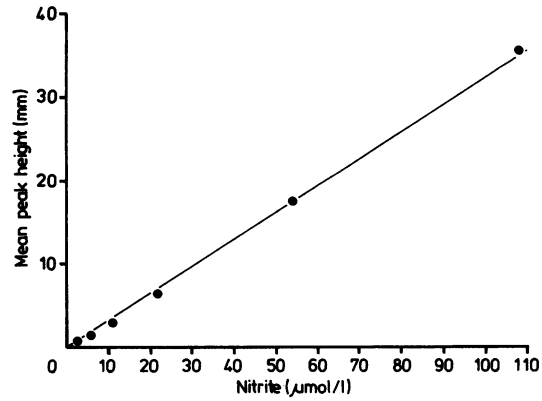
Results

The assay was linear for standard solutions of nitrite (of concentration 7·14 μmol/l–108·7 μmol/l) made up in gastric juice using the peak height produced by the autoanalyser (coefficient of correlation between nitrite concentration and peak height = 0·99) (Fig.). The coefficient of variation ranged from 5·4% (for 14 samples at 5·44 μmol/l) to 0·8% (for 11 samples at 108·7 μmol/l). When gastric juice was spiked with nitrite, sufficient to raise the nitrite concentration by 37·42 μmol/l, the mean percentage recovery was 97%. Saturating gastric juice at pH 7·35 with bilirubin (Sigma) had no detectable effect on the assay.

The mean nitrite concentration and pH were calculated for each patient. The coefficient of correlation for all 85 subjects between pH and nitrite, using the mean values in each patient, was 0·76 (Spearman's Rank test) ($p < 0·01$).

Discussion

The results indicate that the Chemlab cartridge in conjuction with an autoanalyser is a suitable method for the analysis of nitrite in human gastric juice. The



Graph to show linearity of assay for solutions of sodium nitrite of known concentrations made up in gastric juice. (Standard errors of the mean ranged from 0·0 to 0·09 and were too small to be discernible on the graph.)

avoidance of protein precipitation and bilirubin interference without resort to complex equipment is a considerable advantage. The relation between pH and nitrite is in agreement with results obtained using different methodology.[1-3]

In conclusion, the suggested role of nitrite in human gastric cancer has led to a series of studies on gastric juice nitrite. We believe that the method described provides a simple and rapid analytical technique suitable for performing such studies.

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Letters to the Editor

Effect of an evacuated blood collection system on coagulation screening tests

An evacuated blood collection system for routine blood sampling is now being introduced into many British hospitals as a labour and cost saving technique.[1] The use of these evacuated tubes for coagulation studies, however, has been severely criticised and even condemned.[2,3] In most cases it is not convenient or desirable for a hospital to have to maintain a second routine blood taking method just for coagulation investigations. We have therefore compared samples drawn with venous cannula and syringe with those obtained by an evacuated tube technique.

Blood specimens were collected from 30 apparently healthy adult volunteers and from 19 patients regularly attending an anticoagulant clinic, who were selected at random. Venous blood was obtained by clean venepuncture with a 21 G butterfly needle (Argyle; St Louis, USA) and a disposable polystyrene syringe. Nine volumes of blood were added to one volume of 0·105 M trisodium citrate in a polystyrene plastic tube (Brunswick; Sherwood Medical, Co Antrim, N Ireland). Blood was also drawn through the same 21 G butterfly by means of a multiple sample luer adapter into a sterile siliconised evacuated tube (Vacutainer; Becton Dickinson, London) containing 0·105 M buffered sodium citrate. The present study was performed with one batch of Vacutainer tubes, no 676624, lot no I E 114, before their expiry date. The blood was immediately centrifuged at 2000 *g* for 15 min and the platelet poor plasma was tested within 60 min of venesection. Both types of tubes remained stoppered until tested. The thrombin and prothrombin times (using the Manchester comparative reagent)[4] and the activated partial thromboplastin time (APTT) with kaolin[5] were measured in duplicate on each sample by standard manual techniques. The differences between the results of the three tests performed on plasma obtained by the two sampling techniques in the normal and anticoagulant groups are shown in the Table.

Differences between results of the coagulation tests on plasma obtained by the two techniques—plastic syringe and tube and an evacuated tube Vacutainer system

Coagulation test	*Normal group (n = 30)*			*Anticoagulation group (n = 19)*		
	Mean difference	*SD*	*p*	*Mean difference*	*SD*	*p*
Thrombin time (s)	−0·55	0·94	3·31	−0·50	1·01	0·05
Prothrombin time (s)	0·13	0·62	0·24	−0·11	1·06	0·67
Activated partial thromboplastin time (s)	1·63	1·66	<0·001	3·16	3·23	<0·001

The evacuated tubes gave an acceptable correlation with the plastic tubes for the prothrombin time and thrombin time in both the normal and anticoagulant groups; but there was a significantly longer APTT with the evacuated samples in both groups ($p < 0·001$). The lengthening of the APTT ranged up to 4 s in the normal group and 7 s in the anticoagulant group. This was sufficient to make six of the 30 normal volunteers' APTTs abnormal as defined by our laboratory range. Previous reports using evacuated Vacutainer tubes have shown a shortening of the APTT related to an old stopper formulation containing isoprene[2] and prolongation of the APTT after storage of samples in unstoppered tubes before testing.[6] We used evacuated tubes with neobutyl rubber stopper formulation[7] and performed all the tests from stoppered tubes. The precise cause of the variable prolongation of the APTT which we observed remains uncertain, but it may result from adsorption or inactivation of intrinsic contact coagulation factors.

With several commercial evacuated tubes for blood collection now being widely introduced into hospitals in the UK it is important that their effects on the coagulation system are compared with the standard syringe and polystyrene plastic tube method before they are considered for routine use.

IJ MACKIE
DF BARNARD
C JENKINS
SJ MACHIN
Department of Haematology, Middlesex Hospital Medical School, London W1P 7LD

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