Nitroglycerin analysis: spurious low concentrations with the automated U.S.P. content uniformity assay in the presence of dextrose

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Recent interest in nitroglycerin (glycerol trinitrate, GTN) has been stimulated by its re-emergence as a potential drug of first choice in treating post-myocardial infarction patients (Dean & Baun 1975; Flaherty et al 1975). Since neither 0·9% NaCl or sterile water were suitable vehicles for i.v. administration, 5% dextrose was adopted; but when GTN solutions for i.v. use were prepared for hospital use, an initial significant decrease in concentration was found in the dextrose solutions. This report describes the reason for this apparent decrease.

All reagents were analytical grade unless indicated otherwise. Acetonitrile was from Burdick and Jackson (glass distilled, Muskegon, MI). Standard solutions of GTN were made from GTN spirit standardized against potassium nitrate using the U.S.P. phenoldisulphonic procedure (U.S.P., 1975a), GTN solutions for assay were prepared by diluting GTN spirit to 7·5, 15 or 22·5 μg ml^{-1} with 0, 1, 3, or 5% dextrose in water.

The U.S.P. content uniformity procedure (U.S.P., 1975b) for the analysis of GTN was used as described by Bell (1964) with the following modifications. The concentration of *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) was reduced to 0·25 from 1 mg ml^{-1} to stabilize the baseline, the number of samples per hour was reduced to 20 to allow better separation between samples and the ratio of sample to wash was 1:4. Three standard samples were run at the beginning of each series and one standard was run after every 10 samples. The sensitivity of the assay was 5 μg ml^{-1}. The method revealed an initial significant decrease in GTN concentration which was repeated subsequently. To determine if this was related to dextrose concentration, GTN solutions were prepared as above and assayed immediately. Solutions in 5% dextrose yielded apparent GTN concentrations about 15% less than those in water at each GTN concentration. Increasing the dextrose concentration led to decreasing GTN concentration, indicating that the problem was definitely related to the dextrose concentration.

Increasing the concentration of the assay reagents and the temperature proved futile while dextrose assayed alone produced no apparent interference with the assay. At this point it was unclear whether the concentration difference was the result of rapid GTN degradation, complexation between dextrose and GTN, or an interference with the U.S.P. assay. For this reason an h.p.l.c. assay was used (Crouthamel et al 1978) which is specific for GTN but adaptable for identifying mono and dinitrate degradation products. Table 1 shows that there is no effect of dextrose on GTN concentration according to the h.p.l.c. assay, nor were any degradation products found. This suggested that the problem was in the assay and was the diazotization step between the nitrate ion and the amine procaine. To elucidate this further, the procaine concentration was greatly increased (20X), with some improvement in results, with the 3 ng ml^{-1} standard showing an 18·7% deficiency and the 50 mg ml^{-1} standard showing a reduction of 6%. This suggested that the dextrose and nitrate ion were competing for reaction with the procaine molecule. Thus, there was a decrease in the amount of procaine available for diazotization, and consequently less of the diazotized procaine compound available for complexation with NEDD. Such an interaction between dextrose and procaine has been suggested by Charonnat et al (1954) who also observed a decrease in procaine local anaesthetic activity in the presence of dextrose.

Table 1. Comparison of an automated U.S.P. content uniformity and h.p.l.c. assay method with varying dextrose concentrations.

GTN (μg ml^{-1})	Dextrose Concn %	Apparent GTN U.S.P. method	concn (μg ml^{-1})* h.p.l.c. method
7·5	0	7·5	7·5
	1	6·8	7·2
	3	6·6	7·7
	5	6·4	7·6
15	0	15·0	15·0
	1	14·1	14·7
	3	13·3	15·2
	5	12·7	15·4
22·5	0	22·5	22·5
	1	21·5	22·6
	3	20·7	22·4
	5	19·6	22·3

* mean of duplicate analyses

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Reduction of mortality by sulphinpyrazone after experimental myocardial infarction in the rat

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In the Anturan Reinfarction Trial (ART 1978), reinfarctions and, expecially, sudden cardiac deaths were less frequent among patients treated with sulphinpyrazone than among those receiving placebo medication. There is wide, general interest in elucidating the mechanism by which sulphinpyrazone brings about these protective effects. Although they are presumed to be in some way related to its platelet-stabilizing action, conclusive evidence is lacking and exactly how the two phenomena are linked is still very much a matter of conjecture. Various research groups have therefore investigated the effects of sulphinpyrazone on the course of events following acute experimental myocardial infarction in animals. In their initial studies, Kelliher et al and Povalski et al (in press) and Moschos et al (1979) found that the early arrhythmias (ventricular fibrillation, ventricular tachycardia and ventricular extrasystoles) occurring subsequent to coronary occlusions in cats and dogs were less frequent if the animals had been pretreated with sulphinpyrazone. We have studied the effects of the drug on mortality after ligation of the left anterior descending coronary artery (LAD) in the rat.

The operation was performed according to Selye et al (1960) in male rats (Tierfarm Sisseln), 222 ± 2 g, anaesthetized with ether. Essentially, this technique consists in ligating the LAD between the left auricle and the pulmonary cone. If the operation is completed within 30 s, spontaneous respiration recommences after air has been expelled from the thorax by gentle lateral pressure and the wound closed.

A few animals died as a result of haemorrhage during or shortly after the operation. In the present series of experiments, there were four deaths in the sulphinpyrazone group and two among the controls. These animals were not included in the evaluation of the results.

Sulphinpyrazone was administered for three days in a dose of 30 mg kg^{-1} s.c. twice daily, mornings and evenings. The controls received 0·9% NaCl (saline). LAD ligature was performed on the second day of treatment, that is between 1–6 h after the third sulphinpyrazone dose. No attempt was made to relate subsequent mortality with the exact time, after the last dose, of coronary artery ligation. After surgery, i.e. excluding the animals that died during the operation, the sulphinpyrazone group numbered 91 rats and the control group 103. These were composed of 12 separate control and sulphinpyrazone-treated groups with 5–11 rats in each.

The post operative mortality was followed up in both groups over a period of 21 days. In computing the mortality rates, a distinction was drawn between deaths within 30 min of the operation and later deaths. This was done because, according to previous findings (Kane et al 1979; Kenedi & Losonci 1973), deaths occurring in the first half hour are predominantly due to arrhythmias, whereas, in our own experience, the main causes of subsequent deaths are pulmonary oedema, hydrothorax or massive infarctions with dilatation of the heart. During the observation period, mortalities were recorded daily; autopsies were performed on the animals that died later than 30 min after operation.

The surviving animals were killed on the 21st day. The hearts were removed, the atria and the right ventricles dissected at the septal junction, and the creatinine phosphokinase (CPK) contents of the left ventricles determined according to Kjekshus & Sobel (1970). The magnitude of the infarcts was estimated from the amounts of CPK released. Allowance for changes in enzyme content due to the surgical trauma were made on the basis of the contents found in the left ventricles of 24 sham-operated rats (exposure of the heart without LAD ligation). In addition, 22 large large infarcts were excised and the CPK contents of this tissue determined.

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