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LETTERS TO THE EDITOR

Interference of chemoluminescence with [^{3}H]scintillation counting*†

Tracing the passage of [^{3}H]chlorpromazine‡ into the tissues and excreta of various mammals, several scintillators were evaluated for the counting of solvent extracts of the labelled drug content from the biological materials, or of the unextracted homogenates of the specimens. When quaternary amine solubilizers, such as Hyamine (Beckman) or Soluene (Packard) were employed to dissolve tissues, especially keratinous material such as wool, fur or nails, high spurious counts were obtained for the unextracted solutions with toluene-type scintillation cocktails. These keratinous tissues had previously been found to accumulate relatively large amounts of chlorpromazine metabolites (Forrest, Forrest & Roizin, 1963). These drug derivatives were of unknown structure, could not be extracted from the alkaline tissue homogenates by solvents, and could be assayed as a group only by destructive methods. Hence, scintillation counting appeared to be the method of choice to establish quantitative criteria for this apparently significant auxiliary drug detoxication mechanism. However, control specimens from animals not having received any labelled drug yielded false positive counts approximating 10 000 to 26 000 counts/min for 50 to 100 mg samples of wool or fur, completely obscuring the small amount of radioactivity expected in the experimental samples. Obviously, the spurious counts were due to persistent chemoluminescence, which could be decreased only moderately at the rate of 10 to 30% by prolonged storage of the samples, lowering of the temperature or neutralizing some of the excess alkalinity, short of precipitating the dissolved keratinous material.

The problem was solved by using the Packard Model 300 Tri-Carb Sample Oxidizer for combustion of the wool or fur samples. This procedure yielded background counts of 25 to 37 counts/min for the controls, while the experimental specimens produced low but unequivocal counts of 10 to 20 times background. Simultaneously, quenching due to the yellow to brownish colours of the dissolved keratinous samples was avoided.

Similar chemoluminescence phenomena were encountered in the scintillation counting of unextracted biological materials such as serum, urine, faeces and tissues of experimental animals. No such interference was observed, when solvent extracts from the alkalinized materials were subjected to scintillation counting, using extraction and sample preparation procedures previously reported with any type of scintillator, even in the presence of Soluene (Bolt, Forrest & Serra, 1966; Bolt & Forrest, 1967; Forrest, Bolt & Serra, 1968; Brookes & Forrest, 1969). As new extraction procedures were to be evaluated, it was essential to establish completeness of extraction of the labelled drug fraction by radioquantitation of the various solvent extracts obtained at different pH's as compared to homogenates of the unextracted material. It was then established that unextracted faeces and tissues or their homogenates showed chemoluminescence comparable to wool or fur, and that treatment in the Packard Oxidizer should precede normal scintillation counting.

The spurious results obtained with various unextracted mammalian urines in the

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Table 1. *Effect of chemoluminescence on scintillation counting in two cocktails.*

Species	Material Unextracted 100 λ	Scintillator I (counts/min) Cycle 1	Scintillator I (counts/min) Cycle 2	Scintillator II (counts/min) Cycle 1	Scintillator II (counts/min) Cycle 2
Rabbit Dutch Belt male	Urine, control	108 963	97 194	37	37
Guinea-pig pigmented, short-haired female	Urine, control	5062	4639	36	34
	Urine, after ^{3}H-CP$^+$, 100 μCi				
	Day 1	7355	5281	4206	4160
	Day 10	5388	4010	236	248
	Day 16	3174	3091	35	36
	Day 62	946	824	33	35
Sheep Strong-Ramble A female	Urine, control	65 342	58 914	37	35
	Urine, after ^{3}H-CP, 2 mCi				
	Day 1	85 222	80 082	11 083	11 072
Rhesus monkey male	Urine, control	26 520	25 696	35	34
	Urine, after ^{3}H-CP, 64 μCi				
	(3-day pool)	15 225	13 361	1,014	977
female	Urine, control	5566	4467	32	33
	Urine, after ^{3}H-CP, 64 μCi				
	(3-day pool)	9997	8537	1164	1156
male	Serum, control	57	54	36	36
Human male	Urine, control	28 786	26 337	33	32
female	Urine, control	6168	5323	32	33
male, neonate	Urine, control	13 996	11 684	37	36

Counted in Packard Tri-Carb Scintillation System, efficiency 33–39%
Scintillator I: PPO 5 g; dimethyl POPOP 0·3 g; Soluene (Packard) 40 ml; toluene to 1000 ml.
Scintillator II: naphthalene 100 g; PPO 5 g; dimethyl POPOP 0·3 g; dioxane 730 ml; toluene 135 ml; methanol from 35 to 100 ml.
$^+$ ^{3}H-CP = tritiated chlorpromazine.

presence of a quaternary amine solubilizer (Soluene) in one of the scintillation cocktails (Scintillator I) are listed in Table 1. This indicates that the use of Scintillator II—which tolerates the presence of 3 to 12% water, according to methanol content of 35 to 100 ml—eliminates all false positive counts. Spurious counts ranging from 10 000 to 100 000 counts/min routinely occurred in control urines, when Scintillator I was employed, but were reduced to background values either by acidification or by substitution of Scintillator II. These measures were mandatory, since Scintillator I also yielded exaggerated counts in experimental samples, and simulated excessively prolonged excretion of the label in e.g. guinea-pig urine.

Persistent chemoluminescence in an alkaline scintillation medium as an interfering phenomenon in scintillation counting has also been reported in different contexts by other laboratories. Kahlbehn (1967) attributed the spurious counts to the interaction of Hyamine 10-X with such tissue components as proteins and polysaccharides. More recently, Woods & O'Bar (1970) described a new type of chemoluminescence arising from the combination of trichloroacetic acid and bathophenanthroline, as distinct from the analogous phenomenon due to reaction of quaternary amines with peroxides.

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A comparison of adrenergic α-receptors by the use of *NN*-dimethyl-2-bromo-2-phenylethylamine (DMPEA)

There has been much discussion of the possible subclassification of the α- and β-categories of adrenergic receptors (cf. *inter alia*, van Rossum, 1965; Lands, Arnold & others, 1967; Patil, 1969; Bristow, Sherrod & Green, 1970; Furchgott, 1970; Brittain, Jack & Ritchie, 1970; Patil, Patil & Krell, 1971; Triggle, 1971). Evidence in support of such subclassification comes from the discovery of tissue selective agonists and antagonists, the comparative sequences of activities of agonists and antagonists in various tissues and by the use of isomer activity ratios (Patil, 1969; Patil & others, 1971) in which the activities of *R* and *S* isomers of catecholamines are compared in a number of tissue systems experimentally controlled to minimize the influence of non-receptor catecholamine processes. This latter method has the theoretical advantage also in that problems of diffusion and access to the receptor, which may complicate comparisons between structurally dissimilar molecules, should be minimized. According to this procedure, the α-receptors of six tissues, including rabbit aorta and rat vas deferens, are not distinguishably different.

This finding agrees with our previous limited report (Moran, Triggle & Triggle, 1969) that the kinetics of recovery of response of the rabbit aorta and rat vas deferens to noradrenaline from irreversible antagonism by *NN*-dimethyl-2-bromo-2-phenylethylamine (DMPEA) were identical. This procedure would appear to afford another potential probe of comparative α-receptor structure, sharing the advantages of the isomer ratio technique, since recovery from an established blockade is measured and the product of the presumed receptor hydrolysis ($Ph{\cdot}CHOH{\cdot}CH_2{\cdot}NMe_2$) is inactive.

Table 1 presents our data, together with brief experimental details, for four α-receptor containing tissues. The results with rabbit aorta and rat vas deferens confirm our previous findings (Moran & others, 1969) and those with the guinea-pig and rabbit vas deferens extend them and show differences in the kinetics of recovery of α-receptor response.

It has been argued elsewhere (Belleau, 1958; Triggle, 1965, 1971) that the recovery of response from alkylation by DMPEA is consistent with a spontaneous intramolecular (direct nucleophilic or general base catalysed) hydrolysis of a β-dialkylaminocarboxylate ester. For such a reaction a decreased rate would be anticipated on shifting to a less polar environment since this would hinder charge production.