In summary, we find that cyclic AMP phosphodiesterase activity always results in a decreased activity in specific tissues of alloxan-diabetic rats, whereas cyclic GMP phosphodiesterase may be increased or decreased in activity. These results support the view that separate enzymes with discrete regulation are responsible for the degradation of cyclic AMP and cyclic GMP in certain mammalian tissues and that the action of insulin on different tissues is highly specific.

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A comparison of 3 reagents in converting thiamine to thiochrome in the presence of plant extracts and polyphenols

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Summary. Assays of thiamine added to plant extracts, known polyphenols and reducing agents, using $K_3Fe(CN)_6$, $HgCl_2$ and CNBr showed that the CNBr method was the least susceptible to redox interference and gave the highest thiochrome yield.

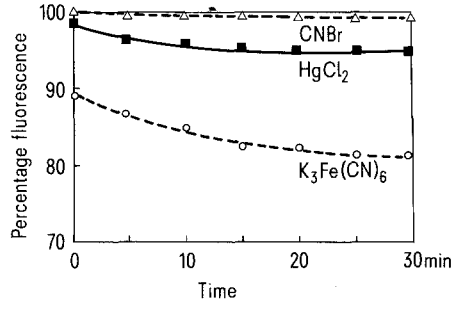
As a result of our research into the thiamine-modifying ability of polyphenolic compounds, we have found the widely used potassium ferricyanide reagent to be particularly susceptible to redox interference by polyphenols[2,3]. This kind of interference is a serious yet not fully appreciated problem caused by compounds, capable of oxidation-reduction, that are not significantly or easily extracted into the isobutanol organic solvent layer, and thus do not interfere with the thiochrome fluorescence as such. The lack of proper appreciation is exemplified by a statement about the ferricyanide assay 'If the sample extracts give low (fluorescence) blanks, the Decalso step may be omitted –'[4]. Since we have good reason to doubt the general validity of the statement[2,3], we have been testing 2 other reagents, $HgCl_2$ and CNBr for their liability to interference by polyphenols and other compounds. Thiamine added to plant extracts (usually rich in polyphenols) and known polyphenols has been assayed using $K_3Fe(CN)_6$, $HgCl_2$ and CNBr and the thiochrome fluorescence in the isobutanol extract compared with that produced from thiamine alone.

Materials and methods. Plant materials were treated and extracted as specified in the AOAC Handbook[5]. Some extracts were diluted before the assay to make them more manageable and freer from interfering substances. Polyphenols and reducing agents of good grade were purchased. The reagents ($K_3Fe(CN)_6$, $HgCl_2$ and CNBr) were prepared and used as described in the appropriate recipes[4,6,7]. Pre-extraction was done by twice vortexing an equal volume of isobutanol with the slightly acidified thiamine and/or polyphenol-containing solutions for 5 min. Fluorescence measurements were performed on an Amino Bowman spectro-

Table 1. Percentage relative fluorescence of added thiamine in plant extracts as developed by $K_3Fe(CN)_6$, $HgCl_2$ and CNBr. The standard errors vary from about 1 to 5%

Reagent	$K_3Fe(CN)_6$		$HgCl_2$		CNBr	
Treatment	I	II	I	II	I	II
Kapok seeds	92	97	51	52	91	94
Betel nut kernel	3.6	19	1.9	4.0	73	94
Betel leaves	n.d.	10	n.d.	44	n.d.	97
Peanut	78	96	60	72	101	99
Tomato	23	85	12	36	43	91
Lemon	1.4	92	3.8	64	63	94
N. oleracea, Lour	12	14	1.1	12	60	94
Rice bran	20	24	22	9.6	85	91

n.d. = not determinable because the extract became too viscous; I = plant extracts, II = plant extracts after pre-extraction by isobutanol.



Percentage relative fluorescence readings of thiochrome from eluates of the loaded tea infusion (1 g/50 ml) kept at 85 °C for various times. Here 1.25 ml of the infusion was loaded on to the Decalso column and 5 ml of the eluate was used for assay by the 3 reagents. If only 0.25 ml of the brew was used for column loading all 3 assays yielded the CNBr curve.

fluorometer with 375 nm and 425 nm as the excitation and emission wavelengths respectively. Fluorescence values due to endogenous thiamine were subtracted before percentages were calculated. Absorbance measurements, for inner-filter-effect, were carried out on a Perkin-Elmer Coleman 55 spectrophotometer.

Results and discussion. Table 1 shows the comparative results from assays using $K_3Fe(CN)_6$, $HgCl_2$ and CNBr on extracts of kapok seeds, tomato, lemon, peanut, betel nut, betel leaves, rice bran and *Neptunia oleracea,* Lour (a local edible plant). Without pre-extraction by isobutanol the percentage fluorescence readings were generally lower than those obtained from pure thiamine solutions. This is partly attributable to the inner-filter-effect because the OD_{375} of the isobutanol layer was quite high (> 0.1) in all cases. When the extracts were pre-washed by isobutanol, CNBr gave excellent percentage fluorescence yields in all cases followed by $K_3Fe(CN)_6$ and $HgCl_2$. Not only do the data show the superiority of CNBr in thiochrome formation but also the relatively low solubility of acidified thiamine in isobutanol. With pre-extraction and the resulting insignificant OD_{375} and OD_{425} of the final isobutanol extract used for fluorescence measurements, the lower percentage fluorescence in $HgCl_2$ and $K_3Fe(CN)_6$ assays indicate interference from redox and other causes in the aqueous layer.

In order to identify some compounds in plant extracts for their interfering ability, we focused our attention on known polyhydric phenolic compounds and reducing agents found in plants. Table 2 shows the approximate concentrations of these compounds that began to interfere with the 3 reagents used to assay thiamine. Again, CNBr is the least susceptible, followed by $HgCl_2$ and $K_3Fe(CN)_6$. To provide some idea of the extent of interference, tannic acid at 1 mM gave 0.7% relative fluorescence with $K_3Fe(CN)_6$, 1.3% with $HgCl_2$ and 100% with CNBr. The same order of percentage fluorescence was given by many other polyphenols at 1 mM.

To demonstrate the considerable significance of the results obtained above, we present below our attempted reproduction of a reported experiment, which previously led to the conclusion that brewing of tea could significantly lower the added thiamine contents over the first 10 min[8]. In our hands, with the loading of smaller and smaller amounts of the infusion on to the column, the isobutanol extract of the eluate gave higher and higher percentage fluorescence with all 3 reagents. The figure shows the results from an experiment in which the CNBr and $HgCl_2$ assays show hardly any change in thiamine with time, whereas the $K_3Fe(CN)_6$ assays indicate an artifactual rapid initial change[2]. Thus, contrary to previous findings, our conclusion is that in fortified tea infusion only slight thiamine modification occurred and this was caused by heat and not by the polyphenols in the tea.

Despite interference, added amounts of thiamine in known polyphenol solutions or tea extract showed a linear fluorescence vs concentration relationship with all 3 reagents. The fluorescence/concentration slopes follow the order CNBr > $HgCl_2$ > $K_3Fe(CN)_6$. Finally, although the CNBr reagent gives consistent and reproducible results, more care should be exercized in its preparation and handling.

Table 2. Approximate concentration of polyphenols and reducing agents at which interference began to develop with $K_3Fe(CN)_6$, $HgCl_2$ and CNBr as the assay reagents

Polyphenol and reducing agent	Reagent CNBr	$HgCl_2$	$K_3Fe(CN)_6$
Caffeic acid	80 mM	100 µM	5 µM
Catechol	1 mM	50 µM	
Catechin	20 mM	100 µM	
Chlorogenic acid	1 mM	200 µM	
Tannic acid	5 mM	20 µM	
Methylgallate	2 mM	100 µM	
Hydroquinone	20 mM	100 µM	20 µM
Ascorbic acid	40 mM	2 µM	200 µM
Cysteine	50 mM	600 µM	60 µM

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Karyotypes of several frogs from Korea, Taiwan and the Philippines[1]

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Summary. Rana amurensis coreana, R. plancyi chosenica, R. latouchii, R. narina and *Ooeidozyga laevis* have 2n=26 chromosomes, *R. kuhlii* has 2n=22, and *Kaloula picta* has 2n=28. Males of *R. narina* have a conspicuous heteromorphic pair, No. 8, which might play a rôle in sex-determination.

Karyological data on the anurans of the Far East are very scarce, except for Japanese species[3-5]. The present paper reports the karyotypes of 7 anuran species belonging to the genera *Rana, Ooeidozyga* and *Kaloula.* The former 2 genera belong to the family Ranidae and *Kaloula* to the Microhylidae.

The chromosome spreads were prepared from bone marrow cells according to Omura's method[6]. Relative length and arm ratio of chromosomes were measured on each of 10 metaphase figures. Secondary constrictions were included in, and small satellites were excluded from, the measurements. Chromosome pairs were numbered in order of decreasing mean relative length. Chromosome morphology was described as by Levan et al[7].

Rana amurensis coreana (Seoul, Korea): 2n=26 with 5 large and 8 small pairs. Nos 4, 8, 10, 12 and 13 are submedian and the other pairs median. No. 9 has a secondary constriction on the long arm.