A search for nitrosamines in East African spirit samples from areas of varying oesophageal cancer frequency

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SUMMARY Following the report of the presence of nitrosamine-like substances in samples of home-made spirit from Zambia, which had been obtained from an area where cancer of the oesophagus is common, samples of distilled alcoholic drinks were collected throughout western Kenya and southern Uganda from areas where the frequency of cancer of the oesophagus varies from very common to very rare. The 44 samples of spirit were screened by polarography and substances giving a similar response to nitrosamines were indicated at levels as high as 21 ppm. Subsequent analysis by gas chromatography for selected individual nitrosamines showed no evidence for the occurrence of methylethyl nitrosamine (MEN), diethyl nitrosamine (DEN), dipropyl nitrosamine (DPN), ethylbutyl nitrosamine (EBN), dibutyl nitrosamine (DBN), nor of N nitrosopiperidine (N Pipn), but traces of compounds having a similar retention time as dimethyl nitrosamine (DMN) were observed. However, subsequent examination by mass spectrometry showed no evidence of dimethyl nitrosamine. There was no apparent association between the levels of unknown constituents indicated by polarography and gas chromatography, nor between either of these levels and the frequency of cancer of the oesophagus. The results by gas chromatography and mass spectrometry confirm that polarography is too unspecific to be a useful indicator of nitrosamines.

Cancer of the oesophagus shows dramatic variation in frequency within East Africa. In western Kenya it is the commonest type of cancer in men (Ahmed, 1966; Ahmed and Cook, 1969), whilst a few hundred miles away in parts of Uganda and Tanzania it is hardly ever seen (Davies, Knowelden, and Wilson, 1965; Eshleman, 1966; Williams, 1966; Buckley, 1967; Cook and Burkitt, 1971). The local pattern of variation in East Africa reflects the global distribution of cancer of the oesophagus which shows a greater range of incidence throughout the world than any other widely occurring cancer (Doll, 1967).

Various studies have indicated that alcoholic drinks are involved in the aetiology (Higginson and Oettle, 1960; Burrell, 1962; Clemmesen, 1965; Martinez, 1969), but that the mere quantity of alcohol consumed cannot be responsible for the global pattern of incidence (Doll, 1967). In East Africa beer and spirits are widely consumed in areas of both high and low frequency.

Various nitrosamine compounds have caused oesophageal tumours in experimental animals (Magee and Barnes, 1956; Magee and Barnes, 1967), and, following a report from a high incidence area of Zambia that nitrosamine-like substances had been found in home-made spirits distilled from a fermentation of maize husks and sugar (McGlashan, Walters, and McLean, 1968), samples of alcoholic drinks were collected from a range of areas in East Africa where cancer of the oesophagus occurs with moderate and low frequency (Cook and Burkitt, 1970). The samples were collected from the areas around the hospitals shown in Fig. 1 with the help of local medical assistants.

Analysis

The samples were given a preliminary screening by polarography using the methods described for the original Zambian samples (Walters, Johnson, and Ray, 1970) and reduction waves occurred which, if due to nitrosamines, represented concentrations

Fig. 1 Hospitals visited in the course of the survey

1 Naivasha	25 Kapengoria
2 Nakuru	26 Kapsowar
4 Kenya Tea Co. (Kericho)	27 Kabarnet
5 Tenwek	28 Tambach
6 Kaplong	29 Kitovu
7 Nyabondo	31 Mbarara
8 Kisumu	32 Kabale
9 Maseno	33 Mutolere
10 Mwhila	34 Kisiizi
11 Mumias	35 Nyakibale
12 Nangina	36 Ishaka
13 Bungoma	37 Mityana
14 Kakamega	38 Kisubi
15 Makumu	39 Entebbe 'A'
16 Kaimosi	40 Mengo
17 Kendu	41 Nsambya
18 Homa Bay	43 Bombo
19 Kisii	44 Jinja
20 Kilgoris	45 Iganga
21 Migori-Nyanza Mines	46 Tororo
22 Kapsabet	47 Mbale
23 Eldoret	48 Amudat
24 Kitale	49 Moroto
	50 Soroti
	51 Ngora
	52 Kamuli

up to 21 parts per million. However, polarography is known to be non-specific and can give erroneously high results. McGlashan and colleagues have sub-

sequently pointed out the dangers of relying on polarography (McGlashan, Patterson, and Williams, 1970). Analyses were therefore carried out using gas chromatography and mass spectrometry. The samples were initially concentrated on a commercial spinning-band distillation column giving concentration factors ($\times 50$) and at the same time removing the alcohol. The concentrates were then analysed by gas chromatography using specific detectors which enhance the relative response to nitrogenous compounds.

Independent analysis of individual samples would have been excessively time-consuming, and samples were therefore grouped so that those from areas of similar cancer frequency could be treated as composite samples.

All bulked samples giving an apparent indication of nitrosamines by gas chromatography were further examined by combined gas chromatography and mass spectrometry, in which characterization was verified by use of a high resolution instrument (AEI MS 902) to monitor the parent ions of individual nitrosamines. Attention has been confined to the steam volatile lower molecular weight dialkyl

N nitrosamines, and the heterocyclic N nitrosopiperidine. Since the spirits are themselves distillates it is unlikely that non-volatile nitrosamines would be present.

Full details of the analytical methods used and developed at the Laboratory of the Government Chemist will be published elsewhere.

Results and Discussion

Table I shows the apparent nitrosamine content of the spirit samples from the analysis by gas chromatography and mass spectrometry. Recoveries from standard solutions and from spiked samples have varied from 50% to nearly 100%; the figures quoted in Table I assume 100% recovery.

The gas chromatography values quoted assume that any peak with a retention time similar to a nitrosamine is wholly nitrosamine. If when using the instrument at maximum sensitivity no peak of a retention time similar to a nitrosamine was observed, the result is reported as 'not detected' (ND). The peaks for all the nitrosamines except DMN were less than 0.1 ppm or not detected. When a peak of the same retention time as DMN was greater than 0.1 ppm, the bulked sample was examined by mass spectrometry.

Bulk samples A-H were concentrated ($\times 50$) from 25 ml to 0.5 ml. Under optimum conditions the mass spectrometer can detect DMN at a level of 5 ppm

in such a sample concentrate (equivalent to 0.1 ppm in the original sample). Sample 1 was a composite of three samples from a very high incidence area and 150 ml of sample was concentrated to 0.5 ml ($\times 300$); in this case the mass spectrometer was capable of detecting DMN equivalent to 0.02 ppm in the original sample. Samples J and K were individual ones in which 50 ml was concentrated to 0.5 ml ($\times 100$). No DMN was detected in any of the samples.

Table II shows the values obtained during the original general screening by polarography.

The levels of frequency of cancer of the oesophagus indicated in Tables I and II vary from 20 to 25% of all tumours diagnosed in men in the 'high' frequency area to less than 1% in the areas of 'very low' frequency (Cook and Burkitt, 1971; Cook and Burkitt, 1970). There is no apparent association between the gas chromatographic and the polarographic results and no association between the levels of unknown constituents indicated by the two methods and the occurrence of cancer of the oesophagus.

The time lag between receiving the samples and the final mass spectrometry/gas chromatography analysis was 16 months. During this time the samples were stored in a cold room (10°C) in amber-coloured bottles. Polarographic analysis was carried out at the beginning and end of the exercise and no significant differences were observed.

Area	Frequency of Oesophageal Cancer		Sample Components ¹	Apparent Nitrosamine ² Content (ppm measured by gas liquid chromatography)							Mass Spectrographic DMN (ppm)
				DMN	MEN	DEN	DPN	EBN	DBN	N.N.pip ³	
Northern Nyanza	D	High	12, 13, 14, 15, 16 ⁴ , 22, 23, 24	0.4	ND	<0.1	<0.1	ND	ND	ND	ND
Southern Nyanza	C	High-moderate	7, 17, 18, 21	0.5	ND	<0.1	<0.1	ND	ND	ND	ND
Kisii-Kericho	B	High-moderate	4, 5, 6, 19, 20	<0.1	ND	<0.1	<0.1	ND	ND	<0.1	ND
Kenyan Rift Valley	A	Moderate	1, 2, 25, 26, 27, 28	<0.1	ND	<0.1	<0.1	<0.1	<0.1	<0.1	ND
Buganda	E	Low-moderate	29, 32A ⁵ , 38, 39, 40, 41, 43A	0.8	ND	<0.1	<0.1	ND	ND	<0.1	ND
Southeast Uganda	G	Low	43B ⁴ , 44, 45, 46, 47, 52	0.5	ND	<0.1	<0.1	<0.1	<0.1	<0.1	ND
Teso-Karamoja	H	Very low	48, 49, 50, 51	0.5	ND	<0.1	<0.1	ND	ND	ND	ND
Nkole-Kigezi	F	Very low	31, 32B, 34, 35, 36	0.9	ND	<0.1	<0.1	ND	ND	ND	ND
Northern Nyanza	I	Very high	14, 15, 16	0.2	<0.1	<0.1	ND	ND	ND	ND	ND
Kisumu	K	Very high	8	0.2	<0.1	<0.1	ND	ND	ND	ND	ND
Maseno	J	Very high	9	0.2	<0.1	<0.1					

Table I Apparent nitrosamine content of spirit samples

¹Hospital numbers as in Figure 1.

²Owing to a misdirection about the regional grouping samples 22, 23, and 24 were wrongfully included in the northern Nyanza bulked sample instead of in the Rift Valley bulked sample. If the results had not been negative the samples from these areas would have been regrouped and reanalysed, but in view of the levels observed no further action was taken.

³This sample from Kigezi was imported from the Buganda area.

⁴This sample from Buganda was imported from southeastern Uganda.

⁵N-Nitrosamines: dimethyl (DMN); methyl ethyl (MEN); diethyl (DEN); dipropyl (DPN); ethyl butyl (EBN); and dibutyl (DBN).

N. Nitrosopiperidine (N.N.pip³).

Region*							
Nn Nyanza	Sn Nyanza	Kisii and Kericho	Kenyan Rift Valley	Buganda	Southeastern Uganda	Teso-Karamoja	Nkole-Kigezi
Oesophageal Cancer Frequency							
High	High to Moderate	High to Moderate	Moderate	Low to Moderate	Low	Very Low	Very Low
4.7 (8)*	2.4 (7)	2.4 (4)	3.3 (1)	2.8 (29)	2.3 (44)	1.4 (48)	1.9 (31)
2.8 (9)	3.8 (17)	< 1.0 (5)	1.4 (2)	6.8 (37)	4.9 (45)	1.1 (49)	1.5 (32B)
< 1.0 (10)	4.4 (18)	< 1.0 (6)	< 1.0 (25)	2.8 (38)	1.0 (46)	1.3 (50)	1.0 (34)
1.2 (12)	4.0 (21)	3.9 (19)	1.5 (26)	4.7 (39)	< 1.0 (47)	< 1.0 (51)	< 1.0 (35)
3.1 (13)		2.1 (20)	< 1.0 (27)	4.8 (40)	< 1.0 (52)		< 1.0 (36)
< 1.0 (14A)			< 1.0 (28)	3.9 (41)	13.5 (43B)		
1.1 (14B)			21.0 (22)	1.9 (43A)			
2.5 (15)			1.7 (23)	13.0 (32A)			
8.8 (16)			< 1.0 (24)				
Analysis of Bulk Samples							
2.2 (8, 9, 10)			< 1.0 (1, 2, 25, 26, 27, 28)	3.5	3.5	1.8	1.4
2.3 (12, 13, 14A, 14B, 15, 16)	1.0	1.5	23.0 (22, 23, 24)				

Table II General screening of the spirit samples by polarography¹¹The figures given are an unknown mixture of possible nitrosamines and other contaminants calculated as ppm dimethyl nitrosamine.²Comparison by region: Kruskal-Wallis one-way analysis of variance (rank test) $\chi^2_8 = 5.98$ ($P > 0.05$)³Numbers in brackets identify the hospitals from which the samples were obtained (see Fig. 1).

A standard aqueous solution of DMN (5 ppm) was stored for 12 months under similar conditions without apparent deterioration.

As a final verification, a large sample (2,000 ml) of spirit from a high incidence area was collected and analysed within 10 days of manufacture. A portion of the sample was spiked at source (in Kenya) with a standard nitrosamine solution and analysis of this spiked sample indicated no loss of nitrosamines during transportation. A portion (500 ml) of the sample was concentrated to 0.5 ml ($\times 100$) and analysed by gas chromatography and combined gas chromatography/mass spectrometry and as before no nitrosamines were detected. Under these conditions nitrosamines would be detected at a level equivalent to 0.005 ppm in the original sample.

At the same time as the samples were collected methods of preparation and frequency of consumption of different alcoholic drinks in East Africa were investigated by questionnaire. The results of this part of the survey in relation to the frequency of cancer of the oesophagus are being presented elsewhere (Burkitt, Collis, and Cook, 1971).

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