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The Incidence of Antibiotics in Herd and Retail Milks in Soctland 1984–1985

Report of a survey undertaken by the Scottish Food and Drugs Co-ordinating Committee.

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A survey of the incidence of antibiotic residues in herd and retail milk has been conducted by the Scottish Food & Drugs Co-ordinating Committee (SFDCC). Of all milks tested, 4·4 per cent. contained detectable levels of antibiotic residues. The problem is discussed and recommendations for future action are made.

Antibiotic residues in milk result, in the main, from the treatment of cows for mastitis. Mastitis, a disease of the udder, is widespread in this country, and is encouraged by misuse of the milking machine and by poor hygiene. The Report of the Milk Hygiene Sub-Committee of the Milk and Milk Products Technical Advisory Committee (1963)[1] recorded the unanimous view of the medical expert on the Antibiotics in Milk Panel of the then Ministry of Health that the presence in milk of any antibiotics is undesirable because of the possible ill effects on the health of a few individuals in the population.

The recommendation and report followed a survey carried out in 1961 to determine the incidence of antibiotics in wholesale ex-farm milk on its arrival at the creamery. Of the Scottish samples (representing 11·5 per cent. of the milk volume sampled), 9·9 per cent. were found to contain detectable residues ($\geq$0·01 i.u./ml expressed as penicillin) of antibiotics.

The possible health dangers apparent at that time were of three types. Firstly it was realised that the consumption of small quantities of antibiotics in milk could result in some people becoming sensitised. This could render them subject to a severe reaction to later therapeutic doses. Secondly cases of allergic reaction in individuals hypersensitive to penicillin had been reported. These reactions ranged from mild skin rashes to anaphylactic reactions. Thirdly it was feared that strains of penicillin-resistant staphylococci were becoming common and that any illness such organisms could cause in humans would not respond to treatment with penicillin.

Enforcement Practice with Regard to Antibiotics in Milk

It has always been possible for Food and Drugs Authorities to contend that, as any consumer could expect to enjoy milk supplies free from antibiotic residues,

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the presence of any residues of this kind constitute a contravention of Section 2 of The Food and Drugs (Scotland) Act 1956, which provides that food sold must be of the nature, substance and quality demanded.

In 1964 the Government publicly endorsed a test sensitive to concentrations of penicillin down to 0·05 i.u./ml and did not dissent from the view that the presence of antibiotic residues in milk was undesirable. Since then proceedings have generally been brought when residues in excess of 0·05 i.u./ml have been detected. In 1966, the Milk Marketing Board (MMB) introduced a system of price penalties for milk producers whose milk failed this test and in 1976 they reduced the failure level to 0·02 i.u./ml. The lower figure was introduced mainly in response to pressure from cheese, butter and yogurt manufacturers who, by then, had recognised that higher levels of antibiotic residue interfered in the necessary fermentation processes.

The Twelfth Report of the joint FAO/WHO Expert Committee on Food Additives (1968)[2] made it quite clear that the use of penicillin should not be allowed to give rise to detectable levels in human food. It considered that the level in milk should not exceed 0·006 p.p.m. (0·01 i.u./ml) and similar recommendations were made for other antibiotics (Table I). Continuing world pressure, sensitivity of dairy processes to the presence of residues, and the advice of the Veterinary Products Committee have resulted in the Ministry of Agriculture, Fisheries and Food advising enforcement authorities to prosecute cases down to 0·01 i.u./ml. from 1968. The Scottish Milk Marketing Board have been screening at 0·01 i.u./ml. for advisory purposes since 1983. From the start of 1986, they, and the M.M.B., have been penalising suppliers of milk containing residues above this limit.

TABLE I

RESIDUE LIMITS IN MILK, 1968: RECOMMENDATIONS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES FOR ANTIBIOTICS

Antibiotic	*p.p.m.*	*i.u./ml*
Streptomycin and dihydrostreptomycin	0–0·2	
Neomycin	0–0·15	
Erythromycin	0–0·04	
Oleandomycin	0–0·15	
Penicillin	0–0·006	0·01
Nystatin	0–1·1	
Bacitracin		0–1·2
Polymixin B		0–2·0
Tetracycline	0–0·1	
Chlortetracycline	0–0·02	
Oxytetracycline	0–0·1	
Novobiocin	0–0·15	

An examination of sampling patterns in 1984 (excluding samples taken as part of the survey under review) reveals that, of the 44 Scottish District Councils contributing information, 15 had not sampled herd supplies for the presence of antibiotics and 12 had not sampled retail milk for this purpose. It is considered that while a few of the Authorities mentioned above have few or no herds within

their area, others have been reluctant to sample because enforcement practice against lower levels of antibiotic residues has been patchy due to the technical and medical difficulties of satisfying a Court that an offence has been committed.

Effective enforcement can be assisted by knowledge of the current incidence of antibiotics in milk. It has been claimed that over the past few years there has been a considerable reduction in the number of antibiotic failures recorded in the U.K. In 1980/81 the average U.K. failure rate ($\geq$0·02 i.u./ml) was quoted[3] as 1·4 per cent. of all supplies, and in 1983/84 as 0·4 per cent., for the Scottish while Milk Marketing Board (SMMB) the published failure rate was 0·86 per cent. These figures did not appear to be consistent with each other or with the limited results of the Local Authority surveillance programmes. In 1984, to clarify the issue and to provide data for enforcement purposes, the Scottish Food and Drugs Co-ordinating Committee (SFDCC) decided on a systematic survey for the whole of Scotland.

The Survey

The survey was designed to cover both herd and retail milks throughout Scotland. It was conducted during the year October 1984 to September 1985 and involved 53 District Environmental Health Departments and 6 Public Analyst Laboratories.

To cover randomly all milk producers in Scotland, Environmental Health Departments were asked to sample every seventh farm on their registers during the first quarter and to continue this procedure in the following three quarters. This meant that over the period of the survey approximately half of all producers were sampled, some on more than one occasion. In the event a total of 1646 herd samples were received. For the retail survey, the Departments were asked to take randomly one sample per 20,000 of population per quarter. This resulted in 950 samples during the year.

All herd milks were sampled into new, previously unused 250 ml capacity, wide necked, plastic bottles which had been shown, before the trial commenced, not to interfere with the method used for detecting the presence of antibiotics. For each sample a pro-forma was completed giving the volume of milk and the size of the herd the sample represented. The retail samples were submitted in their retail containers.

The analysis of each sample was carried out by the disc assay method as described in British Standard 4285: 1968[4]. Prior to the commencement of the survey the method was externally validated at the six participating laboratories. Full details of the validation exercise are given in the Appendix.

Participants were instructed to report positive results, defined as those in excess of the detection limit (see Appendix, Table VII).

The samples found to contain inhibitory substances are shown by month and by quarter in Table II and are given in the concentration bands in Table III.

The incidence of positive results in herd (4·31 per cent.) and retail (4·53 per cent.) milk samples was similar. The overall incidence was 4·39 per cent. The highest herd milk result was 0·20 i.u./ml and the highest retail milk result was

TABLE II

ANTIBIOTICS DETECTED IN MILK (1984–85)

MILK SURVEY: PERCENTAGE OF POSITIVE RESULTS (BY MONTH)

Month	Total samples	Positive samples	Per cent. positive	Quarterly number positive	Quarterly per cent. positive	Total milk volume *litres*	Positive milk volume *litres*
Herd survey							
Jan	139	3	2·2			159,603	5,010
Feb	142	14	9·9	23	5·62	147,250	18,880
Mar	128	6	4·7			162,389	4,901
Apr	138	7	5·1			213,359	15,444
May	161	3	1·9	12	3·26	201,131	873
Jun	69	2	2·9			90,004	2,300
Jul	167	5	3·0			265,526	6,458
Aug	150	4	2·7	16	3·72	140,237	5,493
Sep	113	7	6·2			110,090	4,221
Oct	170	10	5·9			179,716	9,235
Nov	147	4	2·7	20	4·56	142,998	5,277
Dec	122	6	4·9			138,050	6,864
Total	1646	71	4·31				
Retail survey							
Jan	72	5	6·9				
Feb	85	2	2·4	10	4·18		
Mar	82	3	3·7				
Apr	54	0	0·0				
May	101	3	3·0	5	2·09		
Jun	84	2	2·4				
Jul	94	1	1·1				
Aug	91	1	1·1	6	2·59		
Sep	47	4	8·5				
Oct	100	10	10·0				
Nov	77	7	9·1	22	9·17		
Dec	63	5	7·9				
Total	950	43	4·53				

0·08 i.u./ml. Only 3 of the 2596 samples tested contained non-penicillin inhibitory material.

No monthly pattern was apparent but when expressed quarterly the incidence of positive results in both the herd and retail surveys was clearly higher in the Autumn and Winter quarters than in the Spring and Summer quarters.

The incidence of herd milk giving results of 0·01 i.u./ml or more was 3·5 per cent. (3·3 per cent. for the combined surveys). In 1961 the incidence at this level was 9·9 per cent. The incidence at, or above, 0·02 i.u./ml was 1·64 per cent. The SMMB failure rate at this level was 0·98 per cent. in the same period. The incidence of the higher concentrations ($\geq$0·04 i.u./ml) was greater in herd milk (1·09 per cent.) than in retail milk (0·51 per cent.).

Part of the difference between the SFDCC survey and the SMMB data may be attributed to different sampling and testing methods whilst the remainder is probably not statistically significant. Macaulay and Packard[5] and Haverbeck[6] *et al.* have evaluated several of the methods used for detecting antibiotic residue and have reported varying responses and sensitivies for different antibiotics.

TABLE III

ANTIBIOTICS DETECTED IN MILK

MILK SURVEY: INCIDENCE OF POSITIVE RESULTS (BY CONCENTRATION)

i.u./ml	Herd	Retail	Total	Percentage of total
≥0·04	18	5	23	0·89
0·02–0·039	9	6	15	0·58
0·01–0·019	31	17	48	1·84
0·005–0·009	10	12	22	0·85
Trace–0·0049	3	3	6	0·23

Discussion

Between 1963 and 1984 the volume of milk produced in Scotland increased by 4 per cent. while the number of producers fell from 7481 to 3051. In the light of 20 years of effort to reduce antibiotic residues in milk and the concentration of milk production that has occurred the decrease in the incidence of positive results (≥0·01 i.u./ml) from 9·9 per cent. only to 3·3 per cent. is disappointing.

Contamination of milk with antibiotic residues can happen in a variety of ways. Forgetfulness, lack of communication between milkers, mistaken identity of cows, failure to read instructions, milk equipment contamination and deliberate non-compliance can all occur. A recent survey of clinical mastitis[7] showed that on average 71 cases of clinical mastitis occur each year in every 100 cows. The range of antibiotics used to treat mastitis is extensive[8]. There are approximately 20 different antimicrobials formulated with some 60 different intramammary products of which 76 per cent. contain penicillin. Not surprisingly there is confusion over the milk with-holding times after antibiotic treatment.

Antibiotic residues in milk create a major problem during the manufacture of cheese and yogurt. The minimum inhibitory concentrations found to create difficulties often cannot be detected by current tests so they cannot always protect the manufacturer from the effects of antibiotic on the development of their sensitive fermentation cultures.

It has proved difficult since 1963 to quantify the public health significance of antibiotic residues in milk. The viewpoint expressed by Olson and Sanders[9] in 1975 "that the problem appears to be of such magnitude that would seem to require the co-ordinated effort of all producer co-operative organisations to bring the problem under control . . ." was not shared by Dewdney and Edward[10] in 1984. They were on the opinion that there was little evidence for primary sensitisation though they recognised that there were a few individuals of such exquisite sensitivity that they would respond to sub-microgram quantities of penicillin (1 litre of milk containing 0·01 i.u./ml of penicillin contains 6 micrograms of penicillin).

There does appear to be a small number of people who suffer allergic reactions to milk containing antibiotic residues. It is difficult to distinguish these from cases of milk allergy or lactose intolerance but in any event such people are aware of their problem and avoid milk.

Enforcement Authorities in Scotland are bound by the terms of The Food and Drugs (Scotland) Act 1956. (Similar provisions apply to the rest of the U.K.). They cannot view the public health significance of antibiotic residues in milk in general terms but must ensure that every pint of milk sold for human consumption is not injurious to health, is fit for human consumption and is of the nature, substance and quality demanded. In particular in determining for the purpose of the Act whether milk is injurious to health, regard must be taken not only to the probable effect of that milk on the health of an individual but also to the cumulative effect of milk, substantially of the same composition, on the health of an individual consuming the milk in ordinary quantities.

Thus, even though at the levels of antibiotic residues usually observed in milk (0·01–0·05 i.u./ml for penicillin) problems of toxicity are not known to arise, and although there are no identified health hazards for the general population, the presence of antibiotic residues in milk is regarded by Local Authorities as a serious offence.

Recommendations for reducing the antibiotic residues in milk are discussed in the next section of this report. The situation as it developed in Eire provides useful guidance. There, in 1966, a campaign against antibiotics in milk produced satisfactory results. However, by 1983 the situation was such that data published[11] indicated that in some months up to 30 per cent. of milk samples contained antibiotic residues greater than 0·003 i.u./ml, and 7·5 per cent. contained residue levels over 0·01 i.u./ml.

This revelation produced prompt action. An Advisory Committee of interested parties (Central Government, Local Government, Veterinary Officers, Farmers and Milk Handlers) recommended a three pronged remedy: a vigorous awareness campaign backed up with frequent testing and a severe penalty scheme. By 1984 only 1 per cent. of milk tested had residue at levels over 0·01 i.u./ml and by early 1985 only 0·05 per cent. of samples failed.

It is clear that constant vigilance is necessary to ensure that milk production routines are strictly adhered to if the subsequent milk quality is not to suffer.

Recommendations

The recommendations made in 1963 to reduce the level of antibiotic residue in milk covered all essential points. That they were not successful is self-evident. It would appear from the U.K. experience of the last twenty years and the parallel Irish experience that an Advisory Panel is necessary to co-ordinate the activities of the various interested parties. The primary recommendation of the SFDCC therefore is that an Advisory Committee be established to make, and co-ordinate the implementation of, recommendations that will reduce the incidence of antibiotic residues in milk.

The Committee endorses most of the recommendations made in earlier reports[1,11]. In particular it considers that the milk producer must be made more aware of the problems associated with antibiotic residues and made, by a clearly defined, graded, penalty scheme, to suffer the consequences of his actions. The Committee, however, consider that the ultimate penalty of a prosecution taken under Section 2, or even Section 1, of the Act should only be necessary as a last resort.

From an enforcement viewpoint the SFDCC would be happy to recommend that Environmental Health Departments increase their monitoring and surveillance programmes but feels this would be of only limited value without clear national guidelines, or, better still, legal standards concerning the maximum antibiotic residues permitted in milk both ex-farm and when sold retail for human consumption.

The SFDCC also consider that the Local Authorities have an important role in any Awareness Programmes. Many Districts, especially those with a significant milk output, employ Milk Officers who already visit farms and give advice on hygiene and other aspects of milk production. These Milk Officers could readily participate in an Awareness Programme.

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APPENDIX

External Validation of the Disc Assay Method for Antibiotics in Milk BS 4285: 1968

Introduction

Following a decision of The Scottish Food and Drugs Co-ordinating Committee to conduct a national survey of antibiotic residues in milk, the Association of Public Analysts of Scotland (APAS) has carried out an external validation exercise on the proposed method.

Method

The analytical method was as detailed in BS 4285: 1968. The statistical procedure given in BS 5497 Part 1[12] for a split level experiment was followed. The method was carried out at two levels, approximately 0·01 i.u./ml and 0·02 i.u./ml respectively.

Six laboratories participated in the exercise. Each conducted an internal validation, after familiarising itself with the method, prior to commencement of

the external validation. To improve the data for the repeatability calculation five of the laboratories used two analysts, acting independently, to test the samples.

Four samples of milk were spiked to the following levels of penicillin:

1a . . . . . 0·0095 i.u./ml
1b . . . . . 0·0110 i.u./ml
2a . . . . . 0·0195 i.u./ml
2b . . . . . 0·0205 i.u./ml

The samples were checked and each sample was then filled into 6 × 250 ml plastic bottles and deep frozen. Following distribution the samples were assayed six days after preparation.

Results

REPEATABILITY

Table IV has been set out in the manner prescribed in paragraph 12 of the BS 5497. The inhibition zone diameters obtained are expressed in mm and the zone areas expressed as a percentage of that of the appropriate standard. The diameters of the standards are given in mm.

TABLE IV

VALIDATION OF DISC ASSAY METHOD FOR ANTIBIOTICS IN MILK

ZONE DIAMETERS IN MM AND ZONE AREAS AS A PERCENTAGE OF THE STANDARD

	Levels—zone diameters				Standards		Levels—zone areas			
Laboratory	1a *mm*	1b *mm*	2a *mm*	2b *mm*	0·01 *mm*	0·02 *mm*	1a *per cent.*	1b *per cent.*	2a *per cent.*	2b *per cent.*
1	10·8	10·5	12·1	13·0	12·0	13·0	81·0	76·6	86·6	100·0
2	10·9	11·8	13·8	13·4	11·5	15·0	89·8	105·3	84·6	79·8
	11·5	12·9	13·1	13·6	11·9	13·8	93·4	117·5	90·1	97·1
3	9·3	10·5	12·0	11·3	9·3	12·0	100·0	127·5	100·0	88·7
	9·5	10·8	12·0	12·8	10·0	11·8	90·3	116·6	103·4	117·7
4	9·1	11·5	10·7	12·7	9·2	11·6	97·8	156·3	85·1	119·9
	10·0	10·0	12·3	12·6	11·3	14·1	78·3	78·3	76·1	79·9
5	8·6	7·8	9·3	11·4	9·4	13·3	83·7	68·9	48·9	73·5
	9·2	8·5	11·3	11·4	9·2	13·9	100·0	86·4	66·1	67·3
6	10·7	13·8	12·7	15·0	12·5	14·0	74·5	123·9	82·3	114·8
	11·0	12·0	13·3	15·2	12·9	14·2	72·7	86·5	87·7	114·6

Dixon's test, for stragglers, was applied to the Table IV data as described in paragraph 13 of BS 5497. No stragglers or outliers were found.

The repeatability (r) and reproducibility (R) values, calculated in the manner detailed in paragraph 14.10 of BS 5497, are given in Table V.

The repeatability values indicate the values below which the absolute difference between two single test results, obtained with the same method, on identical test material, under the same conditions (same operators, same apparatus, same laboratories and a short interval of time), may be expected to lie with a specified probability. (In this case 95 per cent.).

TABLE V

VALIDATION OF DISC ASSAY METHOD FOR ANTIBIOTICS IN MILK

CALCULATED VALUES FOR REPEATABILITY AND REPRODUCIBILITY

		0·01 level	*0·02 level*
r,	mm	2·75	2·39
R,	mm	5·70	5·37
r,	per cent.	53·7	34·2
R,	per cent.	88·8	65·2

The values calculated using data expressed as a percentage of the standard are more useful since they utilise standards run on the same plates. The values calculated from the zone diameters are for guidance only.

REPRODUCIBILITY

The reproducibility values given in Table V show the values below which the absolute difference between two single test results, obtained with the same method, on identical test material, under different conditions, (different operators, different apparatus, different laboratories and/or different time) may be expected to lie with a specified probability. (In this case 95 per cent).

The reproducibility found at the 0·01 level, of 88·8 per cent., indicates the difficulty of supporting the FAO/WHO recommendation that the level of antibiotic residue in milk should not exceed 0·01 i.u./ml.

The results demonstrate that to be able to report, to 95 per cent. of confidence, that a milk contains over 0·01 or over 0·02 i.u./ml of antibiotic residue a single test result of not less than 0·019 or 0·033 respectively is required. Put another way, since all laboratories would carry out the determination in duplicate, mean levels of 0·016 and 0·029 i.u./ml are necessary before the analyst can be confident that the antibiotic residue present is greater than 0·01 and 0·02 i.u./ml respectively.

LOSS OF ANTIBIOTIC WHEN FROZEN

The average of the data values expressed as a percentage in Table IV when compared with the concentration of penicillin added (expressed as a percentage of the 0·01 or 0·02 i.u./ml standard) gives a measure of the total loss of penicillin during the exercise.

TABLE VI

LOSS OF PENICILLIN FROM MILK FROZEN IN PLASTIC CONTAINERS

		1a	1b	2a	2b
Average Table IV value	per cent.	87·4	103·9	82·8	95·7
Added antibiotic	per cent.	95	110	97·5	102·5
Average/added	per cent.	92	94	85	93

This loss contributes to the reproducibility. In six days 6–8 per cent. of the penicillin was lost at the 0·01 level and 7–15 per cent. was lost at the 0·02 level.

LIMIT OF DETECTION

The limit of detection for the method was experimentally determined at the author's laboratory and can be derived from the data in Table VII.

TABLE VII

DISC ASSAY METHOD FOR DETERMINATION OF ANTIBIOTICS IN MILK

LIMIT OF DETECTION

		Plate 1	Plate 2	Plate 3	Plate 4
Blank	mm	6·0	6·0	6·0	6·0
0·0025 i.u./ml	mm	7·0	7·1	6·8	6·5
0·005 i.u./ml	mm	8·5	8·3	8·3	7·9

At 1 per cent. and 5 per cent. significance limits the limits of detection for the method are 0·003 i.u./ml and 0·002 i.u./ml respectively. For the purpose of the report therefore positive results are defined as those equal to or greater than 0·003 i.u./ml.

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The Proline Content and Stable Carbon Isotope Ratio of Genuine United Kingdom Honey

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Analysis of 102 samples of genuine United Kingdom honey for proline content and stable carbon isotope ratio gave data almost identical with the corresponding American data.

Honey is a relatively high priced luxury product and is vulnerable to adulteration by addition of cheaper sugars. As indices of such adulteration American workers have proposed the determination of proline content and the stable carbon isotope ratio ($\delta^{13}C$) of the total organic matter of the honey.

Proline is the predominant amino acid of honey and comes mainly from the honey-bee itself. The amount present is related to the extent of manipulation necessary to convert nectar into honey. White and Rudyj[1] measured the proline content of 740 samples of American honey, by reacting with ninhydrin in the presence of formic acid. A reddish colour is produced. Other amino acids scarcely react at all. This method was officially adopted by the Association of Official Analytical Chemists after collaborative study[2] and was used in this investigation.

The stable carbon isotope ratio of honey offers a method of detecting the presence of sugars derived from plants which have a different photosynthetic pathway from that of the plant types to which the honey is supposed to be related. In particular, the difference between the $\delta^{13}C$ of material originating from cane-sugar or high fructose corn syrup (plants which use the Hatch-Slack C_4 photosynthetic pathway), and the remaining majority of plants (which use the more common Calvin C_3 pathway‡) is clearly seen by mass spectrometry. White and Doner[3] studied the carbon isotope ratios of 119 genuine honeys and showed a clear separation of the $\delta^{13}C$ peak of honey ($-26^{o}/_{oo}$) from that of high fructose corn syrup ($-10^{o}/_{oo}$). Measurement precision in relation to these differences is typically better than $\pm 0{\cdot}3^{o}/_{oo}$. The results of the work of White and Doner were conveniently summarised by Krueger and Reesman[4] in the form of a histogram showing the frequency distribution.

The present study of the proline content and stable carbon isotope ratios was undertaken at the request of the Ministry of Agriculture, Fisheries and Food in order to establish data for genuine United Kingdom (U.K.) honey and particularly to relate this to the American data.

‡ A summary of the Hatch-Slack and Calvin photosynthetic pathways is to be found on page 219 of Reference 4.

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Materials and Methods

A total of 102 samples of honey were examined, most of them being obtained directly from bee-keepers. Of these, 37 were supplied by members of the Bee Farmers Association, and came from various counties of England, Wales and Scotland, 21 were supplied by amateur bee-keepers in the county of Avon, 38 were obtained from the Trading Standards Officer for Oxfordshire and 6 samples were from the London area.

Proline was determined by the procedure described in the AOAC Official Methods of Analysis.[5] After developing, the colour must not be exposed to bright daylight, otherwise appreciable fading can occur.

Stable carbon isotope ratios were measured at Harwell using a light elements mass spectrometer, VG Isogas type 602E. The sample (carbon dioxide) was obtained by the combustion, in an initially evacuated line, of approximately 200 mg of honey in a stream of pure oxygen. A second combustion stage using platinum catalyst ensured the highest possible yield of carbon dioxide, which was then separated from the other combustion products (principally water) by differential freezing, using acetone/carbon dioxide and liquid nitrogen traps; any non-condensable gases were pumped away. Finally the carbon dioxide was purified by passage over heated copper/silver/copper.

The results are expressed in terms of difference from the international standard, PDB, according to the usual relationship:

$$\delta^{13}C = \left\{ \frac{(^{13}C/^{12}C)\ \text{sample}}{(^{13}C/^{12}C)\ \text{PDB}} - 1 \right\} \times 1000^{o}/_{oo}$$

Results

The statistical parameters of the results obtained for the *proline content* of English honeys are shown in Table I and are closely similar to the corresponding data of White and Rudyj[1] for 482 samples of American honey in 1974/75. The frequency distributions were also similar, allowing for the fact that the American data is based on five times as many samples.

TABLE I

PROLINE CONTENT (mg/100 g) OF U.K. AND AMERICAN HONEYS

Source	No. of samples	Mean	Range	s.d.	CV
Present Work (U.K.)	102	51·6	17–102	17·3	33·5 per cent.
White and Rudyj[1] (American)	482	48·3	15–139	18·6	38·5 per cent.

s.d., Standard deviation; CV, Coefficient of variation.

The *stable carbon isotope ratios* of U.K. honeys are compared with those of White and Doner[3] in Table II. These workers analysed 84 samples of genuine American honey and also 35 samples of imported honey, which they found to be indistinguishable from the American, giving a total of 119 samples. The

statistical parameters of the U.K. and American results show very close agreement and the frequency distribution was again very similar.

TABLE II

STABLE CARBON ISOTOPE RATIOS ($\delta^{13}C^{o}/_{oo}$ OF U.K. AND AMERICAN HONEYS

Source	No. of samples	Mean	Range	s.d.	CV
Present work (U.K.)	102	−25·5	−21·8, −27·0	0·82	3·20 per cent.
White and Doner[3] (American and imported)	119	−25·4	−22·5, −27·4	0·98	3·86 per cent.

s.d., Standard deviation; CV, Coefficient of variation.

Conclusions

The 102 measurements of proline content and stable carbon isotope ratios establish data for what is believed to be a typical sample of genuine U.K. honeys covering a wide geographical range and variety of different U.K. plant sources. The results were examined for significant groupings on the grounds of the location or plant species but none could be identified. However, it is observed that the results, in total, are consistent with those previously reported by American workers in that they show no significant statistical differences in the distributions of either the proline content or the stable carbon isotope ratios. In respect of the stable carbon isotope tests, the observed spread raises the question of the level at which adulteration by material derived from a C_4 plant ($\delta^{13}C = -10^{o}/_{oo}$) could be positively identified. It is suggested that significant conclusions could be made only when measurements lie beyond the ±2 s.d. range; i.e. for a C_3 based honey adulteration with cane-sugar or high fructose corn syrup would not be detectable until values more positive than approximately $-23^{o}/_{oo}$ are obtained. For a honey which would otherwise have a measured value of $-25^{o}/_{oo}$ this would mean the addition of about 15 per cent. adulterant. The caution is given, however, that this technique can detect only C_4 based adulterants such as cane-sugar or high fructose corn syrup since the addition of C_3 material, e.g. beet-sugar, would not alter the stable carbon isotope ratio.

Acknowledgement

This work was supported by the Ministry of Agriculture, Fisheries and Food and is Crown Copyright.

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J. Assoc. Publ. Analysts, 1986, **24**, 95–104

Veterinary Residues in Foods and Feeds
Part I
Use of Medicinal Additives in Feeds

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The use of medicinal additives such as coccidiostats, antibiotics and growth promoters (including hormones) in animal feeds is discussed. Areas of concern and the scope of current legislation are highlighted.

Background

Agriculture is a multi-million pound industry which makes a significant contribution to the U.K. economy (approx. 2·5 per cent. of Gross Domestic Product). Some 2·7 per cent. of the total labour force is employed in agriculture, using around three-quarters of the total land area of the U.K. We now produce two-thirds of our national food requirements and three-quarters of our total needs of those foods which can be produced under U.K. climatic conditions. Animal products form an important sector of the agricultural and food industries. Livestock numbers on U.K. farms are shown in Table I. Over the last few years there has been a slight fall in the numbers of cattle and poultry with a small increase in sheep and lambs. Output from livestock has given virtual self sufficiency in beef and veal, pork, poultry, cream and eggs, with shortfalls in bacon and ham, mutton and lamb, butter and cheese (Table II).

TABLE I

LIVESTOCK ON U.K. FARMS IN 1985

	Numbers $\times 10^6$	Value £M
Cattle	12·8	1600
Sheep	35·5	500
Pigs	8	850
Poultry	119·2	500
	Total	3450

Animals husbandry on such a large scale requires the support of a thriving and efficient industry producing suitable feeding materials. Figures for the production of compound feeds during 1984 are shown in Table III, and represent a

Paper presented to the Annual Conference of the Association of Public Analysts in Taunton on 25 April 1986.

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TABLE II

U.K. OUTPUT OF LIVESTOCK PRODUCTS

Product	Output *Tonnes* × 10^3	Self-sufficiency *per cent.*
Beef and veal	1040	98
Mutton and lamb	295	71
Pork	764	104
Bacon and ham	213	45
Poultry meat	787	98
Butter	241	66
Cheese	243	70
Cream	73	92
Eggs (million dozen)	1051	100
Skimmed milk powder	354	139

TABLE III

U.K. COMPOUND FEED PRODUCTION IN 1984

	Tonnes × 10^6
Cattle and calf	4·8
Pig	2·1
Poultry	3·3
Others	0·5
	Total 10·7

slight fall in recent years owing to EEC Common Agricultural Policy decisions to impose quota limitations on the dairy industry. Nevertheless, in money terms, the output still represents some £2000M. The three main influences on demand for compound feeds are:

(1) livestock numbers,
(2) weather conditions, which will affect the quality and quantity of grazing as well as the availability of "straights" used in compounding,
(3) performance of competing feeding systems.

The need to increase self sufficiency in the U.K. and, more generally, to increase food production to feed an ever increasing world population, has focussed attention on the need to maximise efficiency in animal husbandry programmes, i.e. to maximise the conversion of feed into usable protein. Improved output from farm animals has been achieved by selective breeding, by improvements in environmental conditions and nutritional knowledge, and by advances in veterinary medicine. Modern farming practices, involving the intensive rearing of animals in restricted accommodation, inevitably increase the incidence and spread of disease, since the conditions could hardly be more favourable for the rapid multiplication of parasites. Hence, there is a need to add medicinal compounds to animal feeds for three main purposes. These are:

(1) **Therapy:** the curing of outbreaks of disease,
(2) **Prophylaxis:** the prevention of outbreaks of disease,
(3) **Growth promotion:** the improvement of feed conversion efficiency and, hence, growth rate.

It has been estimated[1] that approximately one third of all U.K. feeding stuffs contain medicinal compounds licensed for inclusion without a veterinary prescription, whilst only 5 per cent. of feeds contain medicaments for therapeutic use. Mostly, medicated feeds are used in the pig and poultry industries. The therapeutic use of medicinal compounds is obviously essential to cure outbreaks of disease where possible. As such treatment is carried out under the direction of a veterinary surgeon, no problems should arise for animals or humans, particularly as the treatments will be spasmodic rather than continuous. However, residues can occur in animal products when large doses are administered immediately prior to slaughter, or in the milk of animals undergoing treatment.

The use of sub-therapeutic levels of medicinal compounds as part of a continuous feeding programme needs careful assessment and monitoring. Here, the analytical chemist has an important and essential role to play.

For the purposes of this paper, medicinal additives will be taken to include all those substances incorporated into animal feeding stuffs for prophylactic or growth promotion purposes and will encompass antimicrobials, antibiotics, coccidiostats, anthelmintics and anabolic steroids.

Veterinary drugs may also be administered to animals by direct prescription/treatment (e.g. antibiotics, vaccines), by implantation, (e.g. hormones), as well as by incorporation into the feed. It is the latter situation which will form the major concern of this paper.

Prophylactics are added at sub-therapeutic levels to restrict the spread of disease, since once clinical signs of an outbreak become recognised, it may be too late to effect a cure. However, even in the absence of all clinical signs of disease, chemicals with an antimicrobial action have been observed to improve animal growth rates and feed conversion efficiency. Hormones have also been used extensively in this mode although usually by implantation. Some 32 active compounds are currently listed in the Annexes to the Additives Directive of the EEC. These include coccidiostats, anthelmintics, antibiotics, growth promoters and tranquilisers, although one or two compounds can be used for more than one purpose.

Uses of medicinal additives

COCCIDIOSTATS

Coccidiosis is a disease of the intestinal tract of both mammalian and avian species producing diarrhoea, general debility and emaciation, and ultimately death. The organisms responsible are protozoans and belong to the sub-order Eimeriina, especially *E. tenella, E. necatrix* and *E. brunetti*. After an incubation period of 4–6 days, haemorrhage and necrosis of the intestinal tract occurs. The life cycle of coccidia spans 7 days and comprises two asexual cycles and one sexual cycle. Only in the latter stages of the cycle do clinical symptoms associated with tissue damage appear. Thus, the acute phase of infection may have passed before therapeutic treatment can commence. Furthermore, many coccidiostats are active only in the early asexual stages of development. This provides an additional justification for prevention rather than treatment of the disease. Nevertheless, treatment after the appearance of clinical signs of the

disease can still be beneficial, because not all birds in the flock will be affected at the same time. However, sub-optimal dose levels can be worthwhile in that they permit the development of natural immunity whilst preventing a clinical outbreak. Affected animals pass large numbers of *Eimeria* organisms in the faeces and these can remain viable for a long time, so giving rise to further outbreaks of the disease.

A closely related disease prevalent in turkeys is known as blackhead (histomoniasis). The causal agent is *Histomonas meleagridis*, which is transmitted by nematode worms commonly found in the alimentary tract of turkeys. Infestation produces an ulcerative and inflammatory disease of the caecum and liver and the birds become listless, without appetite and produce yellow faeces leading ultimately to death. Nitrofurans are used prophylactically to prevent the spread of the disease. Dysentery is also caused by bacterial infections and it can be treated by oral vaccines, antibiotics or the use of ipronidazole or ronidazole in the feed.

Some of the chemicals used in feeds as coccidiostats are shown in Table IV. The level of incorporation can be as low as 2–3 mg/kg or as high as 200 mg/kg. The table also shows the recommended withdrawal period (3–7 days) required to ensure that no residues remain in the animals' tissues. This (and the significance of the Annex I or II classification) will be discussed below under legislative control. The chemical structures of approved substances vary widely. Figure 1 shows the molecular formula of clopidol (metichlorpindol), which is of relatively low molecular weight and contains a number of active groups that are analytically useful. By contrast, Figure 2 shows the molecular structure of monensin with a much higher molecular weight and few active groups that could be used analytically for detection purposes. The compound does not absorb UV irradiation and is non-volatile. Analytical problems will be considered further in Part II.

TABLE IV

COCCIDIOSTATS IN FEEDS

	Annex	Level of application *mg/kg*	Withdrawal period *days*
Amprolium	1	62·5–125	3
Arprinocid	1	60	5
Clopidol	1	125	5
Decoquinate	1	20–40	3
Dimetridazole*	1	100–200	6
Halofuginone	1	2–3	5
Ipronidazole*	1	50–85	6
Lasalocid	1	75–125	5
Monensin	1	100–125	3
Narasin	1	60–70	5
Nicarbazine	2	100–125	7
Nifursol*	2	75	5
Robenidine	1	30–36	5
Salinomycin	1	50–70	5

* Anti-blackhead preparations.

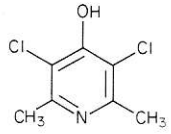


Fig. 1. Structure of Clopidol ($C_7H_7Cl_2NO$).

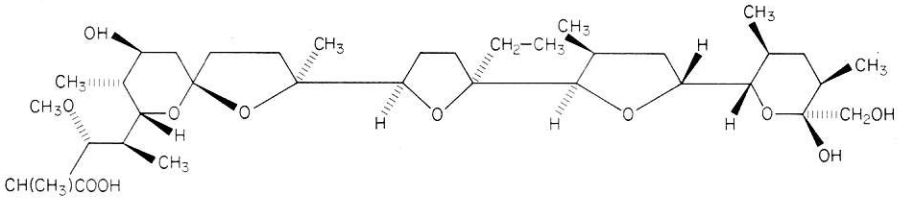


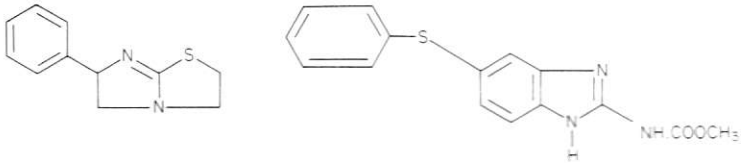
Fig. 2. Structure of Monensin ($C_{36}H_{62}O_{11}$).

The biochemical action of anticoccidial drugs is at present imperfectly understood but the sulphonamides are thought to interfere in the folate synthetic pathway, being analogues of *p*-aminobenzoate which is the basic building block of folic acid. Other compounds may interfere in the metabolism of thiamine (e.g. amprolium) or nucleic acids. The sulphonamides (e.g. sulphaquinoxaline, sulphanitran, sulphamethazine) are water soluble and are active *in vivo* against a broad spectrum of *Eimeria* species. However, the relatively large doses required for therapeutic treatment can lead to symptoms of toxicity. The diaminopyrimidines (e.g. pyrimethamine, diaveridine) are often recommended for use in combination with the sulphonamides as they produce a synergistic effect. Ethopabate is often incorporated into mixtures, since it is active against some of the less common species of *Eimeria*, although it is not active against *E. tenella*. The 4-hydroxyquinolines (e.g. buquinolate, methylbenzoquate and decoquinate) were found to be more active *in vitro* than *in vivo*. Drug resistance is a problem with this group. Other compounds used include the arsenicals (noxarsone, arsanilic acid and arsenobenzene), the nitrofurans (nitrofurazone, furazolidone—particularly active against histomoniasis), amprolium (especially in combination with ethopabate and sulphaquinoxalin as Pancoxin) and the polyether ionophores (monensin, lasalocid, narasin and salinomycin). Halofuginone and robenidine are also used as coccidiostats. Modern practice of protection against coccidiosis involves a programme in which the drugs are rotated between Summer and Winter periods. Ionophores are used in the Summer and chemical products in the Winter, often changing medication every three to four weeks, to obtain maximum protection for the birds. Whilst poultry provide the main outlet for coccidiostats, some compounds have been used in the treatment of ruminants. Cattle and sheep spend a greater part of their lives on dry pasture in widely dispersed herds and are less likely to

become infected than poultry, but where close confinement of animals is practised, outbreaks of coccidiosis have occurred.

ANTHELMINTICS

Anthelmintics (or anti-worm preparations) such as levamisole and various benzimidazole compounds (Figure 3) are widely used for the prevention and control of diseases caused by lungworms, liver fluke, tapeworms and round-worm in cattle, sheep, pigs and poultry. A review of the use of these compounds in the U.K. has been compiled by Watson[2]. Recommended withdrawal periods can vary from 0 to 28 days.



Levamisole Fenbendazole

Fig. 3. Structure of Anthelmintic agents: (a) levamisole; (b) fenbendazole.

TRANQUILLISERS

Tranquillisers are administered to curb the aggressive behaviour of animals and to avoid stress and excitement immediately prior to slaughter. Although frequent use of such drugs is unlikely, the doses required are relatively high and concern has been expressed, since the animals are slaughtered so soon after dosing that residue levels are likely to be significant.

GROWTH PROMOTERS

Perhaps most concern has surrounded the use of other antimicrobial agents such as the antibiotics, and the anabolic steroids used in growth promotion. Whilst few would object to the use of therapeutic doses of antibiotics when necessary to maintain healthy, disease-free stock, it is the administration of low levels of these compounds as growth promoters that has given the greatest cause of concern.

Growth promoters are used to increase the efficiency of meat production either by modification of rumen digestive processes or by stimulation of physiological responses in the animals' body through the use of hormones. The first group of substances (Table V) affect the microflora of the gut in such a way that the production of methane and of volatile fatty acids is reduced. This increases the average live weight gain (kg/day) and decreases the feed conversion ratio (kg feed/kg gain) by comparison with control animals[3] as shown in Table VI. The use of such compounds is controlled by the EEC Additives Directive which specifies the species or category of animal and restricts use up to

a given maximum age only. The maximum concentration of additive permitted varies with the age of the animal in many cases. Some treatments are licensed for use in milk replacer feeds only. No withdrawal period is specified except in the case of nosiheptide; presumably the maximum age specification is sufficient safeguard. Nevertheless, for economic reasons the treatments are widely used. It has been estimated that 75–80 per cent. of the cattle in the U.S.A. are fed feeds containing monensin.

TABLE V

ANTIBIOTICS AND GROWTH PROMOTERS IN FEEDS

	Level of application *mg/kg*
Avoparcin	5–40
Flavophospholipol	1–25
Monensin	10–40
Nosiheptide	1–10
Spiramycin	5–20
Tylosin	5–40
Virginiamycin	5–80
Zinc bacitracin	5–80

TABLE VI

EFFECTS OF RUMEN ACTIVE ANABOLES ON THE GROWTH OF STEERS

Treatment	Dose *g/ton*	Average daily food intake *kg DM/day*	Average daily liveweight gain *kg/day*	Food conversion ratio *kg food/kg gain*
Control	—	8·59	0·92	8·95
Avoparcin	30	8·23	0·94	8·51
Avoparcin	60	8·02	0·99	7·85
Control	—	6·88	1·17	5·88
Monensin-Na	30	6·71	1·25	5·37
Monensin-Na	40	6·81	1·30	5·25

The second group of compounds consists of hormones, or closely related compounds with hormonal activity. They can be classified into:

(1) oestrogens, e.g. DES, hexoestrol, dienoestrol, zeranol, trenbolone acetate and oestradiol,
(2) progestrogens, e.g. progesterone, melengestrol acetate,
(3) androgens, e.g. testosterone,
(4) other compounds such as corticosteroids, prostaglandins and goitrogens

Groups 1 and 2 display female hormone activity whilst group 3 is active in males. The most commonly used compounds are diethylstilboestrol, hexoestrol, zeranol, oestradiol and trenbolone acetate. This dates from observations in the early 1950s, by which it was found that the gonadal hormones were responsible for the differences in growth rates between males and females. Male animals are usually large and have faster growth rates than females as a result of differences

in androgen and oestrogen concentrations. Lower levels of such compounds in castrates in comparison with "entire" animals accounts for the intermediate growth rates observed in steers. Administration of hormones in the feed, or more usually by implantation into the ear, produces a hormonal balance nearer to that found in entire male animals. Furthermore, the use of hormones can also reduce the fat deposition, nowadays so desirable with consumer preference for leaner carcases.

Lamming[4] reported that 30 per cent. of all beef cattle slaughtered in the U.K. have been treated with growth promoting agents, usually in the form of hormone inplants, whereas Ridley[5] speculates that 90 per cent. of British beef cattle are given implants at some time in their life. Such implants (usually in the ear) release a small amount of the active compound over a long period of time and the resulting levels of exogenous androgens and their metabolites in the tissues of treated steers are only marginally higher than the androgen levels in untreated animals. Levels of exogenous oestrogens in the meat of treated steers are much lower than the corresponding levels found in the meat or milk from untreated heifers or cows. Hence, consumption of tissues from treated animals should present no hazards for humans. However in Italy, following a report of abnormal breast development in several baby boys, who had consumed canned baby foods based on veal, consumer concern has been expressed and as a result the legal status of these compounds is currently under review. The levels present in tissues from treated animals will be discussed in Part II of this paper.

Problems with the use of drugs in feeds

Whilst few would question the use of drugs for the treatment of sick animals, addition of sub-therapeutic concentrations of highly active substances on a continuous basis merely for growth promotion purposes is less easy to defend particularly during times of over-production. In 1968 the Swann Committee was set up to review the use of antibiotics in animal husbandry and veterinary medicine with particular reference to the phenomenon of infective drug resistance. Concern over possible dangers arose from the reported increase in the incidence of resistant strains of bacteria following treatment with antibiotics. Further problems followed the discovery that some micro-organisms which are resistant to antibiotics could transfer resistance to other micro-organisms which had not been exposed to the antibiotic concerned. This is particularly important if the receiving organisms are pathogenic. Furthermore, residues of antibiotics, if present, in edible animal products (especially milk, eggs and meat) could cause allergic reactions in sensitive individuals.

Following the publication of the Swann report[6], further restrictions on the use of antibiotics in feeds were imposed.

Legislation

The addition of medicinal compounds to feeds in the U.K. is controlled by the Medicines Act 1968 and supporting Statutory Instruments which implement the EEC Additives Directive 70/254 as amended. This latter piece of legislation treats all chemicals as additives whereas in the U.K., medicinal products are

controlled by the Medicines Act and processing aids or nutritional additives are controlled by the Agriculture Act 1970. The practical effect of this legislation is to prohibit the manufacture, sale and supply of veterinary medicinal products except under licence. Licences are issued by Ministers, who are advised on the safety, quality and efficacy of medicinal products by the Veterinary Products Committee, which consists of appointed but independent experts. Medicinal products currently available in the U.K. are classified into 2 types: (1) Prescription Only Medicines (POM) i.e. products available for use only under the direction of a veterinary practitioner, and (2) Products on the Pharmacy and Merchants' List (PML products), which are available from either registered pharmacists or approved merchants. In addition, there is a General Sale List containing products which may be used in feeds, but few of these are recognisable as true medicinal additives (e.g. sodium bicarbonate for reducing bloat in the rumen).

The EEC Directive 70/524 made a clear distinction, in the case of antibiotics, between their use in "large" quantities for medicinal purposes and in "small" quantities for a physiological nutritional effect. It also divided all medicinal compounds into two categories. Products were included in Annex I only if:

(1) they had a favourable effect on the characteristics of the feed or on livestock production,
(2) they did not endanger animal or human health or the environment,
(3) the levels could be controlled,
(4) they would not affect the treatment or prevention of disease,
(5) for serious reasons concerning human or animal health their use did not have to be restricted to medical or veterinary purposes.

Products could be placed in Annex II for a specified period if all the above conditions could not be satisfied provided that there was no known danger to animal or human health. The Directive also required that feeding stuffs could be placed on the market only if the nature, level and expiry date of any additive were declared. Since that time, the Directive (or, more particularly, the Annexes to the Directive) has been amended over 50 times; the latest amendment being Commission Directive 85/520/EEC of 11 November 1985. The last consolidated version of the Annexes was published as Commission Directive 85/429/EEC of 8 July 1985. Furthermore, the articles themselves were amended by Council Directive 84/587/EEC of 29 November 1984. This contains an additional Annex III, which specifies the minimum conditions which must be fulfilled by manufacturers of additives, premixes and compound feeds. Annexes I and II list the additive, its chemical formula, the species of animal for which its use is permitted (with maximum age, where appropriate) the minimum and maximum contents permitted in the feed, together with other provisions such as withdrawal period or prohibition for use in laying birds.

Compounds with a hormonal or thyrostatic action are not included under the terms of Directive 70/524 and are the subject of a separate Council Directive (81/602/EEC of 31 July 1981). This banned the use of substances having an oestrogenic, androgenic, gestagenic or thyrostatic action for farm animals except under the control of a Veterinary Surgeon for therapeutic use. The use of stilbenes, thyrostatics and their derivatives was banned completely. A decision

on the use of oestradiol-17β, progesterone, testosterone, trenbolone and zeranol for fattening purposes was postponed. This merely reflected the legal status of compounds in Member States at the time and delayed a decision on those compounds for which no agreement could be reached. Following publication of the Directive, specialist committees were established to consider the technical aspects of the problem in greater detail. Subsequently, natural hormones (oestradiol-17β, testosterone and progesterone) were stated to have no harmful effect on the health of consumers, provided that the recommended conditions of use were adhered to. In order to counteract possible unauthorised usage, monitoring schemes were proposed, although it was recognised that abuse would be difficult to detect by the methods of analysis then available. A supplementary Council Directive (No. 85/38/EEC), published on 16 July 1985, requires Member States to undertake random controls as necessary, including the taking of samples from farms and slaughterhouses. Analysis by radio-immunoassay, TLC or GLC was recognised as being definitive until official methods have been agreed by the Commission. However, in October the European Parliament voted by an overwhelming majority to ban the use of all growth promoting substances, both natural and artificial, in meat production. This was confirmed by the EEC ministers in December, although the vote was not unanimous and there is some doubt as to whether it can be legally enforced. Nevertheless, several European countries are demanding assurances that meat imports are accompanied by a statement that no hormone residues are present. Analytical chemists have a major part to play in the enforcement of such controls for the protection of the consumer and problems encountered in the analysis of feeds and foods for medicinal additives will be considered further in Part II of this paper.

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J. Assoc. Publ. Analysts, 1986, **24**, 105–107

Book Reviews

GOOD TO EAT. RIDDLES OF FOOD AND CULTURE. By M. Harris. Allen & Unwin, Price £12.95. ISBN 0 04 306002 1. 248 pp + Bibliography and Index.

Seldom can a book review amount to such a recommendation of sheer entertainment as with this 1985 book now published for the first time in Britain.

There are eleven chapters, dealing essentially with human nutrition, but the writer asks in them why it is that omnivorous man, who is able to eat almost anything, hedges himself around with so many food preferences and prohibitions.

Very often, when men say they are hungry, they are speaking of a hunger for meat. Poland's crisis in 1981 arose because its government threatened a cut in subsidies for meat, yet their total calorie intake and daily protein intake was about the same as in USA, and their total protein intake was 170 per cent. of the WHO daily protein recommendation. The Soviets import soya beans, maize and wheat, but not to feed their human population. They produce ample grain for those. The imports are almost all for feeding their farm animals.

It is only in heavily overpopulated places that the cost benefit of consuming such concentrated food as meat is lost. Thus in Hindu India the cost benefit of preserving their bovine species for traction purposes outweighs the benefit in using land to feed them for their use as food. The prohibition on their use as food was not primarily a religious one. Brahmans once consumed enormous amounts of beef. The convenient religious prohibition was added after the benefits of preserving oxen had been appreciated.

Similarly, in the Middle East, the pig was eaten for as long as the forests remained and the pig enjoyed its own habitat. When the forests were cleared for agriculture, the pig was found to compete for food which man could eat, and the prohibitions in Leviticus postdate the discovery that pigs were less cost effective in the Middle East than goats. When American farmers in a later age first overproduced maize, they used the surplus to raise pigs, and before the Civil War they consumed more pork than wheat. Later, Americans deliberately destroyed the buffalo in order to provide grazing for domestic cows, and the change from droving to the transportation of meat without bone or offal led to the change to a preference for beef over pork.

The reason the horse is not eaten is that it is uneconomical to feed. Its value as a war machine was demonstrated when cavalry first overcame the infantry of Ancient Rome. Islam was horse mounted, and its warriors supplemented their rations by drinking horse blood and consuming the flesh of war casualty horses. Their lightweight horses were only stopped, in AD 732, by heavier French horses carrying knights in armour. That year Pope Gregory III wrote a letter ordering an end to the consumption of horse flesh. Horses were essential for the protection of Christianity!

And thus the book continues, with its message that what man eats is the food which is most cost effective in the region where he consumes it. Our nearer animal cousins, the chimpanzees, enjoy eating insects and they even select rotten fruit to eat because of its content of nutritious maggots. Where it is nutritionally worthwhile to collect them, man also quite cheerfully adds such insects as locusts and waterbugs to his diet. Because of the way modern American beef is fattened there is always an excess of fatty beef, so cost effectiveness in USA has found expression in a prohibition on the use of "Ham" in "Hamburgers".

The book may make public analysts wonder who is pulling what strings. Esquimaux and Lapps eat extremely high animal fat diets yet have lower rates of cardiovascular disease than most groups. They need the fat to help absorb fat-soluble vitamins like Vitamin D, and they recognise a disease caused by consumption of too much low-fat meat such as rabbit.

After reading this book many people will ponder current nutritional advice and may find themselves humming the Porgy and Bess song, "It ain't necessarily so".

A. C. BUSHNELL

MAXIMUM CONCENTRATIONS AT THE WORKPLACE AND BIOLOGICAL TOLERANCE VALUES FOR WORKING MATERIALS 1985. VCH Weinheim, 1985. Price DM 24.

This is a limp 94 pp. edition of an annual revision of maximum concentrations of chemical substances allowable in the air of a working area so as not to impair the health of an employee nor cause undue annoyance.

The values are used in West Germany as a basis for Technical Regulations on hazardous working materials. The book is in English and is well written.

A new and unusual inclusion is a column in the table concerning effects of the various substances listed on pregnant females.

Of interest too is the separate listing of working materials which have been shown to be carcinogenic, and these include beech and oak wood dusts.

Of great interest nowadays is smoking with its carcinogenic effects, and smoking in a workplace, particularly passive smoking, does merit the mention it receives even if it cannot be quantified.

Regrettably no index is given but there is a list of Commission members, together with a short request from the Chairman of the Commission for submission of scientific, technical or other data on the substances quoted.

The book is well printed, informative and of great interest to persons involved in health and safety work and advice, and is certainly worth the reasonable cost.

G. V. JAMES

INSTRUMENTELLE MULTIELEMENTANALYSEE (Instrumental Multielement Analysis). Edited by Professor Dr Bruno Sansoni. Zentralabteilung f. Chemische Analysen Kernforschungsanlage Jülich GmbH. Published by VCH Verlagsgesellschaft D-6940 Weinheim, Federal Republic of Germany, 1985. Price DM 160 (approx. £48). 782 pp.

This recently published book gathers together papers on nuclear radiation spectrometry, activation analysis, ICP source mass spectrometry, X ray fluorescence analysis, atomic fluorescence spectrometry, forward scattering, voltammetry and ion chromatography. Ten articles describe sample preparation, standards, and data evaluation, while 26 papers present applications of multi-element analysis.

The cover, preface and about one third of the papers are written in German while the remainder are in English. This will not encourage the non-German-speaking reader to delve deeper into the volume, which is a pity.

The practising analyst will be disappointed, for there is little attention paid to actual details of techniques; for example, Atomic Absorption is allocated three pages, and Ion Chromatography ten pages (in German), and the general impression is that the papers are concerned with research grade instruments and techniques which are well beyond the immediate reach of most laboratories.

Nevertheless, there is no doubt that many of the techniques described will become well-established and commonplace in the near future, and for that reason the book will not begin to date as rapidly as some of the Development Reviews published over the last decade.

D. J. TAYLOR

J. Assoc. Publ. Analysts, 1986, **24**, 109–110

ERRATA

J. Assoc. Publ. Analysts, 1986, **24**, 59–68

Arachis Oil: A Re-investigation of the Evers Test

M. J. WALKER AND D. THORBURN BURNS

Two mistakes occured in the above paper: (1) a value was omitted in the tabulated composition of arachis oil on p. 59 and Figures 1 and 2 appeared with the wrong captions. The correct Table and Figure layout are shown below.

Fatty acid	*Per cent.*
Oleic acid, $C_{18:1}$	50
Linoleic acid, $C_{18:2}$	28
Palmitic acid, C_{16}	10·6
Stearic acid, C_{18}	3·8
Behenic acid, C_{22}	3·1
Arachidic acid, C_{20}	1·7
Lignoceric acid, C_{24}	1·6
Eicosenoic acid, $C_{20:1}$	1·2

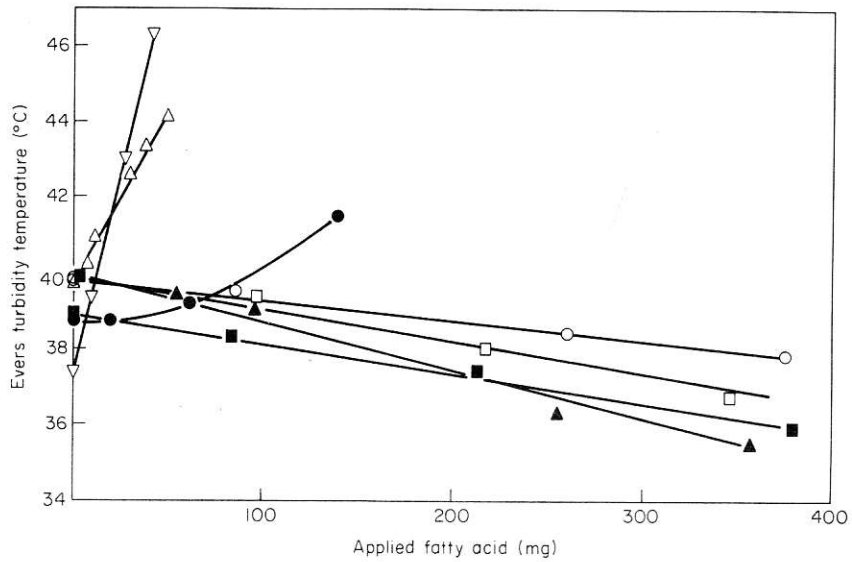

Fig. 1. Influence of added fatty acids on the observed Evers turbidity temperature. ●, Arachidic acid; ○, linoleic acid; ■, oleic acid; □, palmitic acid; ▲, stearic acid; △, behenic acid; ▽, lignoceric acid.

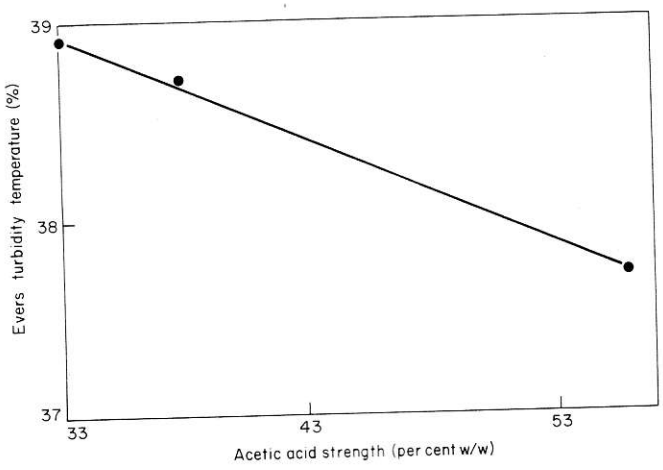

Fig. 2. Influence of strength of acetic acid used on the observed Evers turbidity temperature.