Volume 23 Part 3 September 1985

JOURNAL
OF THE
ASSOCIATION OF PUBLIC ANALYSTS

The Connective Tissue Content of Four Bovine Cuts

DAVID W. LORD AND KAREN J. SWAN

Lancashire County Analyst's Department,
Pedders Lane Road, Dock Estate, Preston

Data on the composition, particularly the connective tissue content, of trimmed bovine shin, flank, diaphragm and masseter muscle are presented.

Public Analysts and enforcement officers serving the Northern English and Welsh Counties of Cheshire, Clwyd, Cumbria, Greater Manchester, Gwynedd, Lancashire, Merseyside and Shropshire have co-operated in a survey of the composition of four bovine cuts. The survey was co-ordinated by the authors and compositional analysis was undertaken in the laboratories of the Greater Manchester County Analyst, the Lancashire County Analyst, the Merseyside County Analyst, and of Messrs. Ruddock & Sherratt.

The four cuts examined were chosen because they were cheaper bovine cuts and were known to be used in the manufacture of comminuted meat products.

Two of the cuts, shin and flank, are of skeletal muscle recognised as beef by the consumer and purchased at retail level.

The other two cuts, diaphragm and masseter muscle, were formerly regarded as offal[1], but now are referred to in the 1984 Meat Products and Spreadable Fish Products Regulations[2] as "parts of the carcase which are to be regarded as meat".

At retail level, diaphragm can be purchased for human consumption (as skirt) but cuts of masseter muscle (with associated head tissue) are generally retailed only for animal consumption.

This aspect of consumer usage is important in considering the use of bovine masseter muscles in comminuted meat products. For example, beefburgers are not traditionally made from this poor quality cut, and the mere legal transition from offal to "part of the carcase to be regarded as meat" does not imply that beefburgers can be made solely from masseter muscles. In our opinion, a beefburger should contain no more than a small proportion of its lean meat as lean bovine masseter and should be prepared mainly from cuts of beef recognised by the consumer as such.

The survey was particularly aimed at establishing data on the connective tissue content of the lean part of the cuts although other useful data have emerged.

Connective tissue is defined as in a previous paper[3]:

$$\text{Wet-fat-free-connective tissue} = \text{Hydroxyproline} \times 37$$

0004–5780/85/030071 + 05 $02.00/0

Sampling and Sub-sampling

Sampling and sub-sampling methods were designed so that "Lean meat" was prepared for analysis.

The aim was to produce, by trimming, lean meat close to the consumer understanding, but perhaps biased a little in favour of the manufacturer, in leaving the epimysium intact.

The internal and external masseter muscles were cut from bovine heads by experienced butchers. The muscular tissue with associated fat was trimmed free of papillous tissue, salivary glands and lymph nodes. Laboratories received the trimmed muscles from the butchers.

Observation of the trimming procedure indicated that no significant amount of muscle tissue would be removed with the fatty, glandular and papillous tissues.

Approximately 5 lb samples of shin, diaphragm and flank were submitted to laboratories. The samples comprised lean meat with attached fatty tissue. Fatty tissue was trimmed to produce lean meat.

To ensure consistency in sub-sampling the four laboratories held a joint demonstration of trimming procedures.

Sub-samples were passed through mincers using progressively smaller cutting grilles (at least twice) followed by homogenisation in a chopper/blender in preparation for analysis.

One hundred and seven samples of the bovine cuts were analysed from home-produced animals.

Methods of Analysis and Quality Assurance

Each laboratory used its normal operational methods for determination of water, ash, fat (acid digestion procedure), nitrogen and hydroxyproline. There were slight procedural differences between laboratories other than for hydroxyproline for which all laboratories used the BS 4405 Part II method[4].

Each laboratory was required to adopt a minimum within-laboratory quality control rate of 20 per cent. during the survey, i.e., twice the minimum rate recommended by the Association of Public Analysts[5]. This minimum was to be exercised as replication quality control, i.e., one in five determinations were to be replicated but laboratories were advised, in addition, to continue their normal recovery experiment quality control procedures[6]. The acceptability of quality control data was assessed by individual laboratories by their usual procedures.

This level of within-laboratory quality assurance was supplemented by a small programme of between-laboratory quality assurance. Three limited cooperative/collaborative trials were carried out to assist in monitoring the quality of analytical data, namely, on two occasions a material was circulated amongst the four laboratories for nitrogen determination and on a third occasion a material for hydroxyproline determination.

The results of these trials are shown in Tables I and II.

TABLE I

NITROGEN CONTENTS OF BEEF CUTS IN COLLABORATIVE SURVEY

	Lab 1	Lab 2	Lab 3	Lab 4
Mean nitrogen content found (*per cent.*)	15·74	15·66	15·62	15·56
Intra-laboratory standard deviation*	0·07	0·07	0·04	0·06
Difference from overall mean as percentage of overall mean	+0·6	+0·1	−0·1	−0·5

* Minimum of eight replicates by two analysts over two circulations of survey samples.

TABLE II

HYDROXYPROLINE CONTENTS OF BEEF CUTS IN COLLABORATIVE SURVEY

	Lab 1	Lab 2	Lab 3	Lab 4
Mean hydroxyproline content (*per cent.*)*	0·48	0·50	0·50	0·48

*Each the mean of four determinations by two analysts.
Reproducibility[6] was calculated to be 0·05 per cent. of hydroxyproline.

In the opinion of the authors, the trial data indicate acceptable between-laboratory variance.

The summarised analytical data for each of the bovine cuts are shown in Table III.

Wet-fat-free-connective tissue content has been calculated as indicated earlier and connective tissue levels for each group of samples are shown.

Discussion

The trimming of the cuts of flank, shin and diaphragm in laboratories was designed to avoid removal of epimysium and hence could be argued to produce meat which was a little higher in fat and connective tissue content than if trimmed by the consumer.

The cautious trimming procedure may have left excess fat on certain of the trimmed sub-samples and this is suspected in several samples. Nevertheless data from all the samples have been included in the evaluation of the composition of lean meat.

Table III clearly indicates that the "lean" portions of shin, flank and diaphragm would have connective tissue contents (as defined) on average of around 10 per cent.

Furthermore none of the 80 samples of the three cuts had a connective tissue content above 18 per cent.

This table also shows that masseter muscles have considerably higher connective tissue content than the other cuts, with a mean of 19·2 per cent. (range 7·2 to 28·5 per cent.).

TABLE III

THE COMPOSITION OF FOUR BOVINE CUTS

Cut	Number of samples		Water *per cent.*	Fat *per cent.*	Nitrogen *per cent.*	Hydroxyproline *per cent.*	Wet-fat-free connective tissue* *per cent.*	Nitrogen on fat-free meat *per cent.*
Masseter	27	Range	66–76·2	2·4–14·9	3·07–3·72	0·20–0·77	7·4–28·5	3·30–3·84
		Mean	72·9	5·2	3·36	0·52	19·2	3·54
		Standard deviation	2·9	3·2	0·14	0·14	5·4	0·15
Shin	26	Range	72·1–77·4	1–3·7	3·20–3·77	0·17–0·45	6·3–16·7	3·28–3·88
		Mean	74·6	2·5	3·51	0·32	11·6	3·60
		Standard deviation	1·3	0·7	0·13	0·08	2·8	0·14
Diaphragm	27	Range	60·7–74·5	4·6–20·9	2·74–3·57	0·1–0·45	3·7–16·7	3·19–4·30
		Mean	69	10·5	3·15	0·22	8·2	3·52
		Standard deviation	3·6	4·3	0·22	0·1	3·7	0·24
Flank	27	Range	61·9–75·3	1·2–20·4	3·04–4·13	0·09–0·48	3·3–17·8	3·38–4·46
		Mean	70·3	7	3·49	0·25	9·2	3·75
		Standard deviation	3·2	4	0·25	0·08	2·8	0·24

*Wet-fat-free-connective tissue = per cent. hydroxyproline × 37.

If masseter muscle were to form a small proportion of the lean meat content of a meat product, say 10 or 15 per cent., then an increase in the connective tissue content of the product (expressed on the lean meat content) of around 1 to 4 per cent. would be evident.

In general the authors' earlier recommendation[3] of a 10 to 20 per cent. allowance for the connective tissue content of beefburgers is seen from the current data to be reasonable if applied to beef products made from the bovine cuts surveyed.

Table III also shows nitrogen levels in the lean meat expressed on the fat-free meat content. The Analytical Methods Committee recommends an average nitrogen factor of 3·55 "as the best compromise for general use"[7].

The table clearly supports the use of a factor close to the recommended value.

References

1. Food Standards Committee, "Report on Offals in Meat Products", HMSO, London, 1972.
2. "The Meat Products and Spreadable Fish Products Regulations 1984", SI 1984, No. 1566, HMSO, London, 1984.
3. Lord, D. W., and Swan, K. J., *J. Assoc. Publ. Analysts,* 1983, **23**, 131.
4. British Standard Methods of test for Meat and Meat Products, BS 4401, Part 11, Determination of L(−) Hydroxypyroline content, BSI, London, 1979.
5. Lord, D. W., *Analyt. Proc.,* 1984, **21**, 394.
6. "Precision of test methods", BS 5497, Part I, BSI, London, 1979.
7. Analytical Methods Committee, *Analyst,* 1963, **88**, 422.

J. Assoc. Publ. Analysts, 1985, **23**, 77–81

Some Factors which may Influence the Variation in Proximate Composition and Nitrogen Factor of Turkey Muscle

T. C. GREY*, D. ROBINSON* AND J. M. JONES

AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA

A study of the proximate composition and nitrogen factors (NF) of breast, thigh and drumstick muscle from 18 week female Wrolstad and Sun Valley II + turkeys has been made. Statistically significant differences in NF of thigh and drumstick meat between the two breeds were found. Storage of uneviscerated Wrolstad turkeys at 4 and 7°C between 1 and 15 days resulted in a significant increase in NF of breast and thigh. No difference was found in composition between the two storage temperatures. Similarly no difference was found between wet- and dry-plucked Sun Valley II + turkeys.

A previous study of the nitrogen factors (NF) of meats from 24-week-old turkey stags (SVII and B.U.T. 6 strains) indicated that there was a significant difference between air- and water-chilled turkeys (Grey *et al.*[1]). It was also established that the NF for thigh and drumstick from present-day hybrids differed from that previously published for leg muscle, obtained from birds of unspecified history. (Analytical Methods Committee[2]). It was thought that this difference could be due to the breed, the earlier published values being obtained from the slower growing poults available in the 1960's.

The purpose of the present study was to establish if this supposition was correct and also to examine the effects of other factors such as wet or dry plucking and storage on the proximate composition and the NF of breast, thigh and drumstick muscles. The latter two muscle groups were included rather than leg muscle in order to evaluate the contribution of each to the NF of leg muscle.

Materials and Methods

Twelve Sun Valley II + female turkeys were removed from a commercial growing house at 11 weeks of age and reared on deep litter in the Institute's animal house until killed at 18 weeks of age. The birds were electrically stunned and killed by neck dislocation; six were hand plucked, and the other six were scalded at 50°C for 3 min. All were chilled at 4°C for 24 h. They were then sampled after evisceration by removing breast, thighs and drumsticks.

Twenty-nine Wrolstad female turkeys were grown in a commercial growing house until killed on the farm at 18 weeks. They were killed by electrical stunning followed by neck dislocation, hand plucked, hung on racks for approximately 3 h and cooled with circulating air at ambient temperature. The

* Present address: AFRC, Food Research Institute—Bristol, Langford, Bristol BS18 7DY.

0004–5780/85/03077 + 05 $02.00/0

birds were then transferred to a 4°C room and held there overnight prior to transportation to the Institute. Two storage temperatures, 4 and 7°C, were used and the birds sampled in the manner described above. The sequence of sampling was as follows. Of the birds stored at 4°C, eight were removed at day 1, five at day 8 and five at day 15. Of those stored at 7°C, five were removed at day 8 and the remaining six at day 15. In all cases the samples were prepared for analysis as described by Grey *et al*[1].

STATISTICAL EVALUATION

Differences between two treatments were statistically evaluated using the Students "*t*"-test.

Results

EFFECT OF BREED ON PROXIMATE COMPOSITION AND NF

The proximate composition and nitrogen factor of muscles from the two breeds of turkey stored at 4°C for a similar period (1 day) are shown in Table I. Thigh and drumstick muscle nitrogen factors were significantly different between the two breeds, being always higher in the Wrolstad turkey. Although the fat content of the thigh muscle of the Wrolstad birds was more variable, the nitrogen factors for the thigh and drumstick muscles were similar. The compositions of breast muscle from the two breeds were identical.

EFFECT OF WET OR DRY PLUCKING

The comparison in composition and nitrogen factors between wet- and dry-plucked Sun Valley II + turkeys is shown in Table II. Although moisture content was consistently lower in wet-plucked turkey, the difference, as with other components, was not statistically significant.

TABLE I

PROXIMATE COMPOSITION AND NITROGEN FACTORS OF MUSCLES FROM SUN VALLEY II + AND WROLSTAD 18 WEEK FEMALE TURKEYS

	Sun Valley II+		Wrolstad			
	mean	s.d.	mean	s.d.	"t" value	p value
Breast						
Nitrogen *per cent.*	3·87	0·04	3·87	0·07	0·06	n.s.
Moisture *per cent.*	73·99	0·58	73·99	0·34	0·01	n.s.
Fat *per cent.*	1·20	0·28	1·24	0·19	0·42	n.s.
Nitrogen factor	3·92	0·04	3·92	0·07	0·08	n.s.
Thigh						
Nitrogen *per cent.*	3·21	0·07	3·28	0·06	2·35	<0·05
Moisture *per cent.*	74·85	0·60	73·00	1·19	4·06	<0·01
Fat *per cent.*	4·41	0·74	6·08	1·45	3·02	<0·05
Nitrogen factor	3·36	0·07	3·49	0·04	5·34	<0·001
Drumstick						
Nitrogen *per cent.*	3·21	0·07	3·39	0·06	6·02	<0·001
Moisture *per cent.*	75·44	0·56	75·20	0·28	1·28	n.s.
Fat *per cent.*	3·21	0·67	3·06	0·42	0·63	n.s.
Nitrogen factor	3·32	0·06	3·49	0·06	6·62	<0·001

Sun Valley II +, $n = 12$; Wrolstad, $n = 8$; n.s. = not significant.

TABLE II

THE EFFECT OF DRY OR WET PLUCKING ON THE PROXIMATE COMPOSITION AND NITROGEN FACTOR OF MUSCLES FROM 18 WEEK SUN VALLEY II+ FEMALE TURKEYS

	Dry plucked		Wet plucked			
	mean	s.d.	mean	s.d.	"t" value	p value
Breast						
Nitrogen *per cent.*	3·86	0·04	3·88	0·03	0·83	n.s.
Moisture *per cent.*	74·19	0·74	73·79	0·30	1·21	n.s.
Fat *per cent.*	1·16	0·24	1·23	0·33	0·43	n.s.
Nitrogen Factor	3·91	0·04	3·93	0·04	0·80	n.s.
Thigh						
Nitrogen *per cent.*	3·24	0·08	3·18	0·06	1·69	n.s.
Moisture *per cent.*	74·98	0·30	74·70	0·82	0·78	n.s.
Fat *per cent.*	4·04	0·51	4·77	0·78	1·90	n.s.
Nitrogen Factor	3·38	0·07	3·34	0·06	1·21	n.s.
Drumstick						
Nitrogen *per cent.*	3·22	0·09	3·21	0·04	0·40	n.s.
Moisture *per cent.*	75·64	0·45	75·24	0·62	1·28	n.s.
Fat *per cent.*	3·21	0·93	3·21	0·35	0·01	n.s.
Nitrogen Factor	3·33	0·07	3·31	0·06	0·43	n.s.

Dry plucked, $n = 6$; wet plucked, $n = 6$; n.s. = not significant.

EFFECT OF STORAGE TIME

The composition and nitrogen factors of muscles from Wrolstad turkey stored for specified periods and the statistical evaluation of the data are shown in Table III. Since no significant effect of temperature was found (see below), the data included for days 8 and 15 were obtained from birds stored at 4 as well as 7°C. Between days 1 and 8 there were significant decreases in moisture content of the breast and drumstick muscles only. No significant differences were found between days 8 and 15 with any of the components. Storage for 15 days resulted in significant differences in nitrogen, moisture and NF between days 1 and 15 in the breast muscle and in the NF of the thigh muscle. The small changes occurring between days 8 and 15 were sufficient to make these differences significant over the longer storage period.

EFFECT OF STORAGE TEMPERATURE

No significant differences in nitrogen factor nor composition were found between Wrolstad turkeys stored at 4°C ($n = 18$) and 7°C ($n = 11$) for neither 8 nor 15 days. Nitrogen factors obtained at 4 and 7°C, respectively were breast 3·96 and 3·97 (s.d. = 0·06), thigh 3·53 and 3·51 (s.d. = 0·06) and drumstick, 3·51 and 3·51 (s.d. = 0·05).

Discussion

Our previous study (Grey *et al.*[1]) showed that B.U.T. 6 and Sun Valley II stags, which were older and heavier than the 18-week Sun Valley II + females used in the present experiments, all had very similar nitrogen factors. The faster-growing Sun Valley II + females (mean liveweight 7·47 kg) and the slower-growing 18-week Wrolstad females (mean liveweight 5·31 kg) used in this investigation have confirmed our view that the difference in nitrogen factor

TABLE III

THE EFFECT OF STORAGE ON THE PROXIMATE COMPOSITION AND NITROGEN FACTOR OF MUSCLES FROM UNEVISCERATED 18 WEEK WROLSTAD FEMALE TURKEYS

	Day 1		Day 8		Day 15		d1 vs d8		d8 vs d15		d1 vs d15	
	mean	s.d.	mean	s.d.	mean	s.d.	"t" value	p = <	"t" value	p = <	"t" value	p=<
Breast												
Nitrogen *per cent.*	3·87	0·07	3·92	0·04	3·93	0·04	2·00	n.s.	0·61	n.s.	2·44	0·05
Moisture *per cent.*	73·99	0·34	73·51	0·48	73·52	0·38	2·46	0·05	0·03	n.s.	2·84	0·05
Fat *per cent.*	1·24	0·18	1·23	0·39	1·30	0·28	0·08	n.s.	0·48	n.s.	0·56	n.s.
Nitrogen Factor	3·92	0·07	3·97	0·04	3·98	0·04	1·84	n.s.	0·76	n.s.	2·38	0·05
Thigh												
Nitrogen *per cent.*	3·28	0·06	3·33	0·08	3·31	0·07	1·48	n.s.	0·43	n.s.	1·23	n.s.
Moisture *per cent.*	73.00	1·19	73·05	0·94	72·37	1·78	0·09	n.s.	1·10	n.s.	0·92	n.s.
Fat *per cent.*	6·08	1·45	5·36	1·13	6·39	2·02	1·15	n.s.	1·45	n.s.	0·38	n.s.
Nitrogen Factor	3·49	0·05	3·52	0·08	3·54	0·04	0·90	n.s.	0·88	n.s.	2·55	0·05
Drumstick												
Nitrogen *per cent.*	3·39	0·06	3·39	0·05	3·41	0·04	0·19	n.s.	1·25	n.s.	1·27	n.s.
Moisture *per cent.*	75·20	0·28	74·64	0·41	74·62	0·71	3·38	0·01	0·06	n.s.	2·41	0·05
Fat *per cent.*	3·06	0·42	3·53	0·84	3·24	0·75	1·56	n.s.	0·84	n.s.	0·68	n.s.
Nitrogen Factor	3·49	0·06	3·51	0·05	3·53	0·03	0·87	n.s.	0·81	n.s.	1·74	n.s.

Day 1, $n = 8$; day 8, $n = 10$; day 15, $n = 11$; n.s. = not significant.

is due to the breed rather than the age or weight. At the moment, having only studied turkey composition to 24 weeks of age, it is not known whether the nitrogen factor increases at a particular age, as was shown with broilers in which the nitrogen factor started to increase from six months of age (Thomas *et al.*[3]). Furthermore, until the other older breeds are studied, it is not known whether or not the differences found are peculiar to the Wrolstad bird.

All three constituents contribute to the variation in NF. The fat content of Wrolstad thigh and drumstick muscles is higher than in Sun Valley II + and the NF has similarly increased. However in the previous study (Grey *et al*[1]), B.U.T. 6 drumstick and thigh muscles had a fat content which was above that of similar muscles from Wrolstad poults, yet the NF was lower and similar to Sun Valley II + in the present study. The interchange between fat and moisture has mainly contributed to the change in NF, although in the case of the B.U.T. 6 the nitrogen content was lower when compared with Sun Valley II +.

Differences in NF can have a profound effect on lean meat content calculations. For example, if the nitrogen content of a sample of thigh muscle were 3·25 per cent. and since

$$\text{fat-free-lean-meat content} = \text{N} \times \frac{100}{\text{NF}} \text{ per cent.},$$

then, since for Sun Valley II + the NF for thigh muscle is 3·36, the calculated fat-free lean meat content would be 96·73 per cent. However, the Wrolstad turkey has a mean NF of 3·49, and the use of this factor would result in a fat-free lean meat content of 93·12 per cent., a difference of 3·62 per cent. The lean-meat content would be even lower if the NF of Wrolstad turkeys (3·54) stored for 15 days was used, namely, 91·8 per cent.

It was thought that the absorption of water during scalding and plucking might affect the value of the NF. Although the mean moisture content in dry-plucked turkey was marginally higher than in wet-plucked, the differences were not statistically significant. Either wet-or dry-plucked turkey data can therefore be used as a baseline.

Conclusions

The nitrogen factors calculated from the proximate composition of thigh and drumstick muscle from Wrolstad turkey are significantly higher than those obtained from Sun Valley II, Sun Valley II + and B.U.T. 6 turkeys.

The nitrogen factors obtained from stored uneviscerated Wrolstad turkeys were only significantly different between 1 and 15 days in thigh and breast muscles and increased with storage time. No differences in nitrogen factors were found as a result of wet or dry plucking or of uneviscerated storage at 4 or 7°C.

The authors thank Mrs. J. Lewis for expert technical assistance.

References

1. Grey, T. C., Robinson, D., Jones, J. M., and Pritchard, R., *J. Assoc. Publ. Analysts,* 1983, **21**, 1.
2. Analytical Methods Committee, *Analyst (London)*, 1965, **90**, 581.
3. Thomas, N. L., Grey, T. C., Jones, J. M., Robinson, D., and Stock, S. W., *J. Fd. Technol.,* 1984, **19**, 11.

J. Assoc. Publ. Analysts, 1985, **23**, 83–91

Determination of Medicinal Additives in Poultry Tissue*

A. Hobson-Frohock

AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA

Tissue residue studies have been carried out on the medicinal additives amprolium, arprinocid, clopidol, dimetridazole and sulphaquinoxaline in broiler chickens and on amprolium and dimetridazole in turkeys. Both gas and high performance liquid chromatographic methods were used and measurable levels were found for all compounds except dimetridazole. The efficacy of the recommended withdrawal period was confirmed. The use of Droplet Counter Current Chromatography and Size Exclusion Chromatography has been suggested to improve sample "clean-up". Application of the Photo Diode Array detector in the field of residue analysis has been discussed and an example given of its use in methodological development.

The analytical work described in this paper was carried out on behalf of the MAFF as the third in a series of contracts concerning residues of poultry feed additives. In the first contract, levels of the antioxidant ethoxyquin were determined in eggs and broiler tissues and in the second the persistence of the coccidiostats decoquinate and sulphaquinoxaline in poultry excreta was investigated when the waste was heat-processed into a feed additive. This work has been published[1,2].

The Ministry has already established a National Monitoring Programme for red meat, mainly concerned with anabolic steroids, and it was proposed that a similar scheme should be set up for poultry meat. The aim of the programme was to determine the level of drug residues in selected tissues of commercial samples and to check that the appropriate "withdrawal period", i.e. the period of application of drug-free feed to the bird before slaughter, intended to minimise the accumulation of residues in the tissues, had been properly applied. The work had three aims which were (a) to establish the methodology to measure residues down to at least 0·05 mg/kg, (b) to feed broiler chickens and turkeys with the selected additives under conditions as close to commercial practice as possible, with and without the withdrawal period, and then (c) to apply the methods to the determination of residue levels in their muscle (leg and breast), liver and, in the case of clopidol, in the body fat.

The additives to be investigated were selected by the Ministry from a list similar to that in Table I, which shows the wide range of compounds available to the poultry producer. It was recognised that the changeable nature of the additives field meant that any compound selected could have gone out of favour with producers by the time the results were available at the end of the two year contract. As far as is known, this did not happen and all the agents investigated

* Text of paper delivered at a meeting of The Association of Public Analysts, 13 April 1984.

0004–5780/85/030083 + 09 $02.00/0

TABLE I

PERMITTED POULTRY FEED ADDITIVES

Growth/egg promoters	
Arsenicals	Nitrovin
Avoparcin	Virginiamycin
Bambermycin	Zinc Bacitracin
Flavomycin	
Coccidiostats/anti-microbial agents	
Amprolium	Halofuginone
Arprinocid	Monensin
Buquinolate	Nequinate
Chlortetracycline	Oxytetracycline
Clopidol	Penicillin
Decoquinate	Robenidine
Dinitolmide	Sulphanitran
Ethopabate	Sulphaquinoxaline
Anti-blackhead drugs	
Dimetridazole	Ipronidazole
Furazolidone	Nifursol
Antioxidants	
Ethoxyquin	

are still in current use, although more attention is now being paid to the ionophors, such as salinomycin, which were not considered in the initial selection.

Methodology

The choice of methodology, i.e., whether to use gas chromatography (GC) or high performance liquid chromatography (HPLC), depended on several factors. HPLC is best suited to compounds of high molecular weight or low volatility, particularly if they are thermally labile. Many therapeutic agents fall into the former category, and for these HPLC tends to be preferred. However, GC was used for clopidol and arprinocid simply because methods were already available in the literature and proved to be satisfactory, albeit with some modifications. The choice of feeding and chromatographic conditions are shown in Table II. The HPLC methods all used reversed phase columns and were generally straightforward. Amprolium was the most difficult because of extraction problems, but these were resolved by the addition of an ion-pair reagent, sodium dioctylsulphosuccinate, to the extracting solvent. Clopidol[3] was converted to the methyl ether with diazomethane and analysed on a packed GC column using an electron-capture detector. Arprinocid[4] was analysed without derivatisation on a wall coated open tubular column (WCOT) using the selective alkali flame ionization detector in the nitrogen mode. This method also used the technique of 'on-column" injection which is rapidly gaining wide acceptance for quantitative work.

One of the major problems with WCOT columns is that where a range of boiling points exist in a mixture to be analysed, it is possible to get discrimination between the lower and higher boiling compounds as they are volatilised from the syringe needle during injection, in both "split" and "splitless" modes. This can

TABLE II

EXPERIMENTAL CONDITIONS FOR DRUG RESIDUES STUDY

	Drug level in feed *mg/kg*	Withdrawal period *days*	Chromatographic method
Broilers			
Amprolium	125	3	HPLC
Arprinocid	60	0*	GC
Clopidol	125	3	GC
Dimetridazole	75	6	HPLC
Sulphaquinoxaline	125	7	HPLC
Turkeys			
Amprolium	125	3	HPLC
Dimetridazole	150	6	HPLC

* Now increased to 5 days.

lead to significant errors in quantitative measurement of the higher boiling fraction and is sometimes called the "syringe" or "needle" effect. It can be overcome by the use of a special injector, whereby a narrow syringe needle is inserted into a cooled section of the actual WCOT column and the sample is deposited directly into the column. Although problems can arise, e.g., from non-volatile residues and the effect of large sample volumes, in general these are not serious and the technique works very well.

The results of the analyses are given in Table III. The values for clopidol warrant some explanation. The feed for these experiments was mixed by a local

TABLE III

RESIDUES OF DRUGS IN BROILER AND TURKEY TISSUES

Drug	Withdrawal period *days*	Tissue concentration *mg/kg*			
		Leg	Breast	Liver	Body fat
Broilers					
Amprolium	0	0·04	0·03	0·35	
	3	<0·01	<0·01	<0·01	
Arprinocid	0	1·32	0·33	0·25	
Clopidol	0	2·53	2·33	9·44	0·24
	3	0·03	0·02	0·36	<0·01
Dimetridazole	0	<0·01	<0·01	<0·01	
	6	<0·01	<0·01	<0·01	
Sulphaquinoxaline	0	1·77	1·87	1·85	
	7	<0·01	<0·01	<0·01	
Turkeys					
Amprolium	0	0·06	0·04	0·38	
	3	<0·01	<0·01	<0·01	
Dimetridazole	0	<0·01	<0·01	<0·01	
	6	<0·01	<0·01	<0·01	

compounder who had specific instructions to exclude all known medicinal additives apart from the normal vitamin/mineral supplement. Unfortunately, at the end of the feeding trial, the control feed was found to be contaminated with clopidol at levels ranging from 0·04 mg/kg in the starter feed to 0·001 mg/kg in the finisher feed. Although these levels are low in comparison with the amount deliberately added to the feed, they were apparently enough to produce a small but measurable residue in the control birds (i.e., those receiving unmedicated feed). As a result, the efficacy of the withdrawal period could only be assessed by comparing the tissue clopidol level after withdrawal with the level in the control birds. The source of clopidol contamination remained unknown although it should be mentioned that rabbit feed containing clopidol was produced on the same site, and aerial contamination could have occurred. There were no such problems with any other additive.

It can be seen from these results that it would be quite feasible to check the proper application of the withdrawal period in the case of amprolium, clopidol and sulphaquinoxaline, although arprinocid would require a further experiment to confirm the efficacy of the withdrawal period. It is understood that it is now the Ministry's intention to use these methods in a survey of commercial samples.

Methodological development

In general, methods for drug residue analysis follow the sequence of extraction, clean-up, separation and quantification, and there seemed to be at least two areas where some improvement was required. It is to these that the rest of this paper is devoted.

(a) SAMPLE "CLEAN-UP"

The first area is that of sample "clean-up" or the removal of those compounds which interfere with the subsequent quantification of the component being studied. Selective detectors can be of considerable assistance in both GC and HPLC[5] methods but inadequate clean-up can still lead to error. For example, electron capture detectors in GC can quickly become contaminated and rendered non-quantitative by undetected material. In HPLC the use of the fluorescence detector with dirty samples can give rise to problems with poor resolution and high column back-pressures caused by overloading with non-fluorescing (and therefore undetected) material. In some cases a simple distribution step between immiscible phases will suffice, particularly if the compound of interest has acidic or basic properties and can be extracted into base or acid. In others, an ion-pair can be formed to increase compound solubility selectively in the organic phase (e.g., in the extraction of amprolium); this technique itself is well worth further study. Another alternative is the use of the small pre-column or cartridge containing HPLC adsorbents (SepPak, Bond Elut etc.). Experience at the Institute has shown that the use of the separating funnel for liquid/liquid extraction is not at all reproducible, particularly where there is a risk of emulsion formation. An interesting alternative is the recently introduced technique of Droplet Counter Current Chromatography[6], shown in diagrammatic form in Figure 1[7]. It is probably best described as the modern form of the Craig Counter Current Distribution Apparatus introduced in 1960[8]

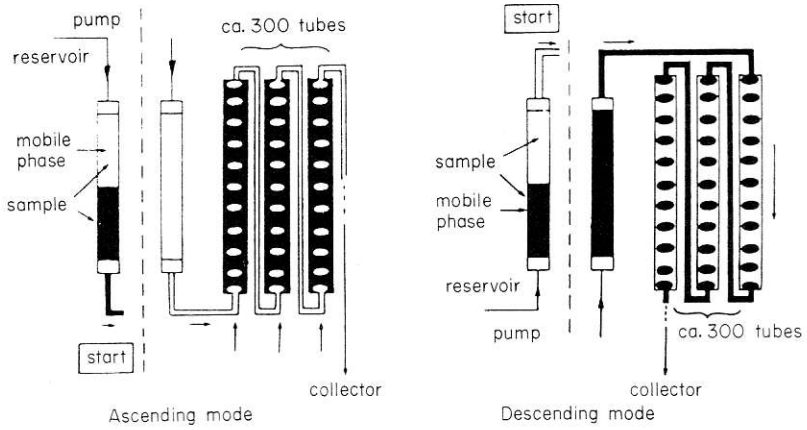

Fig. 1. Schematic diagram of the principle of Droplet Counter Current Chromatography [from Hostettmann, K., *Planta med.*, **39**, 1–18, (1980)].

and widely used in protein analysis though now rarely found in laboratories. It consists of 200–600 tubes in series, each 20 to 60 cm long, 1·5 to 2 mm i.d., connected by Teflon capillaries. The system is filled with a liquid stationary phase, followed by injection of the sample in an immiscible solvent which can be heavier or lighter than the stationary phase. The immiscible mobile phase is then pumped through the tubes, the separation monitored with an appropriate detector (usually u.v.) and the components of interest collected if required. Typical phases are those produced by mixing various ratios of chloroform, methanol and water and taking each of the two layers thus produced. The polarity of the phases can then be adjusted if required. Droplet formation is most important and some systems tend not to produce an adequate number of droplets (methanol–hexane–water, for example). With the absence of any solid support there are no problems with adsorption such as can be encountered with the small packed cartridges and the system can handle quite large amounts of sample from 1 mg to as much as 4 g. The major drawback of poor resolution is not really significant when one is trying to separate a drug of molecular weight around 200 from large amounts of proteins, peptide fragments, lipids etc. To date the author is aware of only one application of the technique and this was reported by Nagata *et al.*[9] who used it to partition acetonitrile extracts of chicken muscle against hexane in the analysis of ethopabate. Somewhat surprisingly, these authors used just three Teflon tubes, 220 × 19 mm i.d., joined together by capillaries 350 mm long, 0·96 mm i.d. The sample volume was 25 ml, injected into 48 ml of *n*-hexane stationary phase with acetonitrile pumped through at 7 ml/min. More than 95 per cent. of the ethopabate was found in the first 20 ml of eluate, so 30 ml were collected and concentrated for analysis. Recoveries of 90 per cent. were claimed at the 0·05 mg/kg level and the method was described as "simple, rapid and sensitive".

Another approach towards improved "clean-up" is the use of Size Exclusion (SEC) or Gel Permeation Chromatography where compounds are separated on the basis of their molecular size as they pass down a column packed with porous spheres. Separation occurs because the smaller molecules can enter the pores

and be retained while the larger molecules enter with increasing difficulty as their molecular size increases. As a result, the larger molecules elute first and the smaller molecules last. The porosity of the spheres can be closely controlled during manufacture to cover a range of molecular size separations. The production of column packings is improving all the time and there are now several excellent materials on the market. The technique is well known in the pesticide field where lipid contaminants are removed on non-aqueous columns but it has not been so readily applied to aqueous extracts. The major advantage is that the nature and composition of the mobile phase is not important provided the sample remains in solution. A disadvantage may be in the reduction of the tissue extract to a volume small enough for application to the column whilst still retaining complete solubility. Preparative columns are available but are extremely expensive. It should be possible to couple an SEC column in series with an analytical HPLC column so that the large molecular weight interfering material would be eluted from the system before the remainder of the extract (containing the compound(s) of interest) was directed through the second column for analysis and quantification.

Another useful modification would be to freeze-dry the tissue before solvent extraction. The major component of raw tissues is water and removal of this before extraction would enable a range of extracting solvents to be examined. Treatment of the tissue with proteolytic enzymes such as Subtilisin A[10] before freeze-drying to prevent losses of the drug "bound" to the tissue would also improve the performance of any method.

(b) THE PHOTO DIODE ARRAY DETECTOR IN HPLC

The second topic relates more to the actual chromatographic separation itself. Chromatography is not an identification technique; it merely separates the components of a mixture. It can be used as an aid to identification of a particular compound but only by matching retention times of the unknown on two or three different columns with those of the substance suspected to be present. This is clearly not easy since one could spend an inordinate amount of time examining known compounds in the hope of finding adequate agreement with the unknown. Identifications are now made by on-line coupling of the chromatographic separation with one of the systems such as mass spectrometry (MS), Fourier transform infra-red (FTIR) spectroscopy or nuclear magnetic resonance (NMR) spectroscopy. These techniques are well known in gas chromatography (GC-MS, GC-FTIR and to a lesser extent GC-NMR), especially with the introduction of benchtop MS instrumentation such as the Hewlett Packard Mass Selective Detector and the Finnegan Ion Trap Detector. However, in HPLC the situation is not so well advanced. HPLC-MS systems are available and, in some research areas, working very well but the technique is not universally available. It is still quite difficult to get much information about the identity of the eluting component, even though the u.v. detector, which could be expected to provide some spectral information towards an identification, is in wide use. Most HPLC detectors only measure the absorption at one particular wavelength (although some models are designed to take measurements at two fixed wavelengths). If a spectrum of the eluting compound is required, the flow of mobile phase must be

stopped in the flow cell during the scanning period. The subsequent loss of resolution usually prevents the collection of the spectrum of more than one compound. The recent introduction by Varian of their Stopped Flow Wavelength Scanning technique is one attempt to overcome this problem. A better solution is to use the PhotoDiode Array Detector[11], shown in outline in Figure 2. Light from a u.v. or visible light source passes through the flow cell and

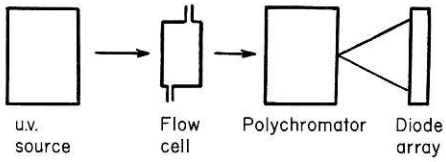

Fig. 2. Outline of Photo Diode Array Detector optics.

into the polychromator where the diodes are positioned such that the full spectrum falls on the linear array. The light incident on the diode sensing area generates a charge which is collected and stored during the integration period. The accumulated charges are then switched sequentially to form the detector output. A complete spectrum can be produced every 10 ms without any disturbance of the mobile phase flow. Several modes of operation are possible and those for the Pye-Unicam PU4021 Multichannel Detector are given in Table IV. The major functions of interest in drug residue work are probably those of

TABLE IV

OPERATIONAL MODES FOR PHOTO DIODE ARRAY DETECTOR

Single wavelength monitoring between 190–390 and 390–590 nm
Dual wavelength monitoring
Programmed wavelength
Sum Absorbance
Peak Purity
Plot Spectra
Spectral/Time Overlay
Library of Spectra for comparison/identification

Sum Absorbance, Peak Purity and Spectral/Time Overlay. In the Sum Absorbance mode, the diode outputs are summed over a pre-selected range and allow the operator to monitor a sample where the absorbance maxima may be ill-defined or unknown. The Spectral/Time Overlay produces a three-dimensional (time vs absorption vs wavelength) presentation the topographical features of which considerably enhance the proper selection of wavelengths for the entire chromatogram (Figure 3). In the Peak Purity mode, spectra can be taken at any point on the eluting peak, e.g., on the upslope, at the maximum and on the downslope. The spectra are then normalised and compared either visually or by the associated data handling system. Any differences indicate the presence of one or more co-eluting components, i.e., an "impure" peak. A typical example is shown in Figure 4 (a,b). In the chromatogram (Figure 4a), the arrowed peak was the compound under test. The spectral/time presentation

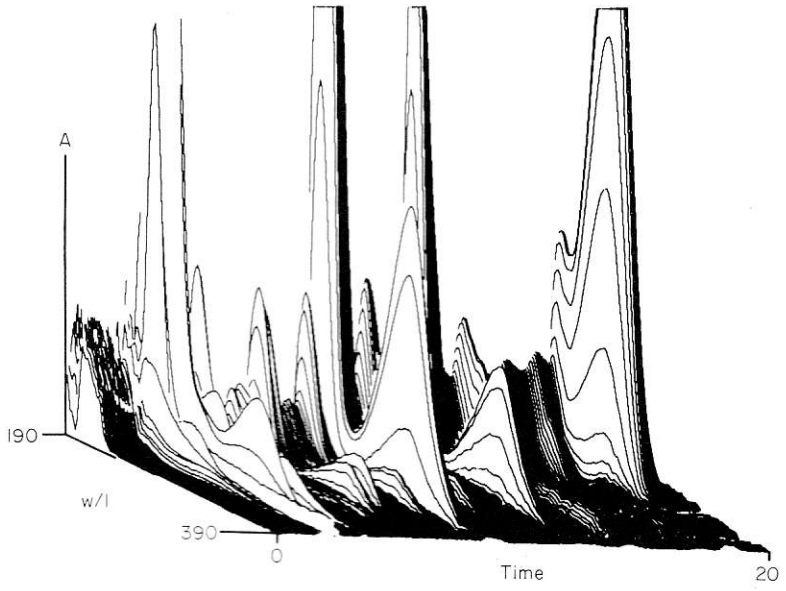

Fig. 3. Three dimensional Spectral/Time Overlay.

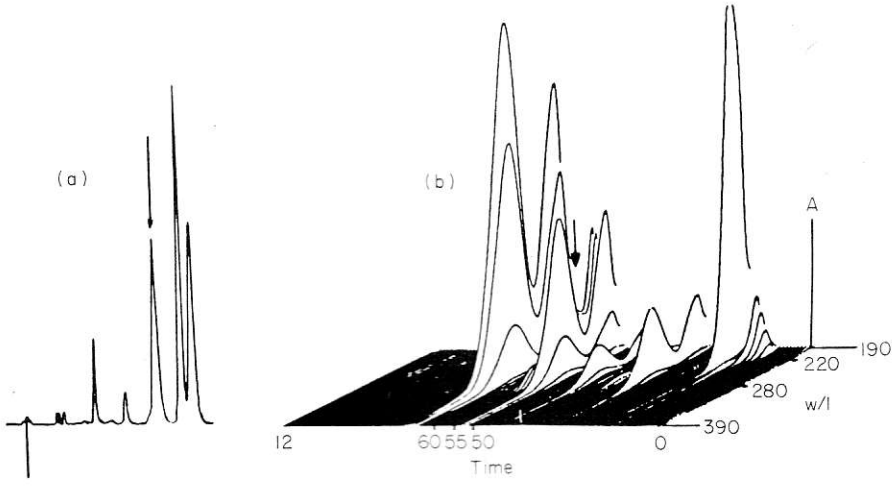

Fig. 4. Detection of "impure peak" using Photo Diode Array Detector. (a) HPLC chromatogram; arrow indicates peak under test; (b) Spectral/Time Overlay; values on time scale (50,55,60) indicate position of individual spectra.

(Figure 4b) indicated a second component at the same retention time. When Spectra 56 and 57 were drawn (Figure 5) the differences were quite clear; the peak in Figure 4(a) was not homogenous and further development of the chromatography was required. The PhotoDiode Array Detector makes compound recognition easier and enables metabolites to be monitored as well as the compounds themselves. Not only does it help to produce a better and more reliable HPLC method but it will be of great value in the development of

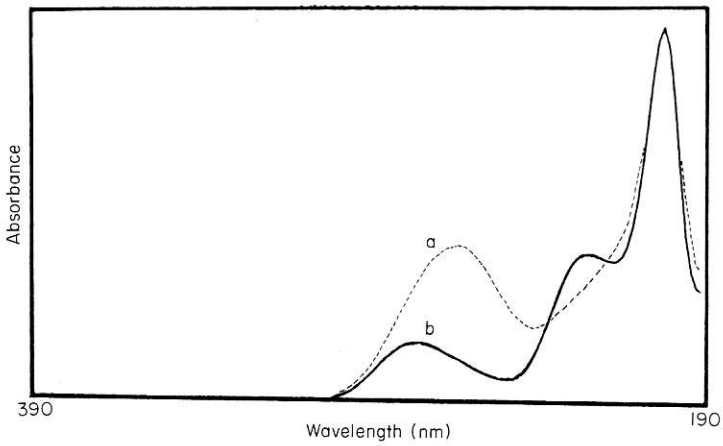

Fig. 5. Spectra of "impure peak" taken from Spectral/Time Overlay. (a) Spectrum number 56; (b) spectrum number 57.

multi-residue methods where one extraction and clean-up would be adequate to quantify several additives of quite different spectral properties. The monitoring of commercial samples can only be economically viable if the particular additive is known; if not, each sample would have to be sequentially analysed for a range of different drugs. Although a single multi-residue method is not likely to be feasible for all known additives, grouping of similar compounds together in one method (e.g., sulphonamides, nitro derivatives) should be possible and should make monitoring of poultry tissues for drug residues a fully practical proposition, a situation which can only be in the consumers' interests.

The work on drug residues was financed by the Food Science Division (MAFF). The author gratefully acknowledges the expert technical assistance of H. A. Johnson, J. A. Reader and members of the AFRC Food Research Institute's Chromatography Development Group.

References

1. Hobson-Frohock, A., *J. Sci. Food Agric.*, 1982, **33**, 1269.
2. Hobson-Frohock, A., and Johnson, H. A., *J. Sci. Food Agric.*, 1983, **34**, 37.
3. Suzuki, E., Matsuda, M., Momose, A., and Namekata, M., *J. Assoc. Off. Anal. Chem.*, 1980, **63**, 1211.
4. Van den Heuvel, W. J. A., and Zweig, J. S., *J. High Res. Chrom. Chrom. Com.*, 1980, **3**, 381.
5. Hobson-Frohock, A., *Analyst*, 1982, **107**, 1195.
6. Tanimura, T., Pisano, J. J., Ito, Y., and Bowman, R. L., *Science*, 1970, **169**, 54.
7. Hostettmann, K., *Planta Med.*, 1980, **39**, 1.
8. Craig, L. C. in "Analytical Methods of Protein Chemistry", Pergamon Press, Oxford, 1960, vol. 1, p. 121.
9. Nagata, T., Saeki, M., Nakazawa, H., Fujita, M., and Takabatake, E., *J. Chromat.*, 1983, **281**, 367.
10. Osselton, M. D., *J. Forensic Sci. Soc.*, 1977, **17**, 189.
11. Overzet, F., Rurak, A., Van der Voet, H., Drenth, B. F. H., Ghijsen, R. T., and De Zeeuw, R. A., J. Chromat., 1983, **267**, 329.

J. Assoc. Publ. Analysts, 1985, **23**, 93–98

A Note on the Composition of some Australian (State of Victoria) Sausages and Sausage Meat

D. MURPHY*

Department of Primary Industry, Canberra, Australia

The Food and Drug Regulations of Victoria, Australia, require that sausages and sausage meat have a minimum meat content of 75 per cent., a maximum fat content of 27 per cent. and not more than 3·5 grains of sulphur dioxide per pound of sausage or sausage meat. Sausage meat not enclosed in a casing and having a meat content in excess of 85 per cent. is not permitted to contain any sulphur dioxide. Pork sausages are not considered separately under the Victorian regulations and must comply with the general standards for sausages and sausage meat.

There have been no recent publications concerning the compliance of sausages and sausage meat with these regulations nor have there been any data published on the distribution of meat and fat contents in those products which do meet the legal requirements. Sausages and sausage meats are the products most sampled by Health Surveyors and represent a significant proportion of the meat consumed by the people of Victoria.

Because of both the lack of recent data on sausages and sausage meat and the significant consumption of these foods, the results on samples of sausages and sausage meats analysed during the years 1976 to 1979 are presented in this paper. Complete data for the years 1975 and 1980 were unavailable but some information from these years is also recorded.

Samples and Methods of Analysis

Sausages and Sausage Meat were purchased by Health Surveyors and submitted for analysis.

The methods of analysis are prescribed by the Food and Drug Standards Regulations[1]. Fat, nitrogen, ash and cereal filler are determined by classical procedures. The nitrogen due to the cereal filler is calculated and subtracted from the total nitrogen to give meat nitrogen. The lean meat content (as a percentage) is then calculated by multiplying the percentage of meat nitrogen by 6·25 to convert to percentage meat protein and then by 4·8, i.e., percentage lean meat = percentage meat nitrogen × 6·25 × 4·8.

* This is an account of some work undertaken whilst employed at Dunn, Son and Stone, Melbourne, Victoria.

0004–5780/85/030093 + 06 $02.00/0

The 4·8 value is an empirically determined protein/lean meat conversion factor.† The sum of the percentages of fat and lean meat then gives the percentage meat content. Fat contents in 1978 and 1979 were determined by means of a Foss-let 15310 fat analyser[2] and all samples which failed to comply with the legal requirements were re-analysed using the prescribed Soxhlet diethyl ether fat extraction process[1].

Sulphur dioxide levels were routinely determined by distillation from an acidified solution of the meat and titration of the sulphur dioxide with standardised iodine solution. If an excess of sulphur dioxide was indicated by this procedure the determination was repeated using the Monier-Williams procedure and the latter result reported.

Results

Table I shows the number of complying and non-complying samples for the years 1976 to 1979.

Table II gives values of the mean, median and standard deviation for the years 1976/1979 of samples of sausages and sausage meat which complied. A minimum meat content of 75 per cent. is required for compliance.

Table III shows values of the mean, median and standard deviation of the fat contents of sausages and sausage meat which complied for the years 1976/1979. The maximum permitted fat level is 27 per cent.

Tables IV and V give respectively the meat and fat content distributions of complying samples for 1976 to 1979.

Table VI contains the non-complying samples for 1976 to 1979.

Table VII gives the available results for samples of sausages and sausage meat analysed in 1975.

Table VIII compares the percentage non-compliance of sausages and sausage meat with manufactured meat, chopped or minced meats and cuts of meat such as chops, steaks, etc.

TABLE I

NUMBER OF COMPLYING AND NON-COMPLYING SAMPLES OF SAUSAGE AND SAUSAGE MEAT

	Complying	Non-complying	Non-complying *per cent.*
1976	1314	173	11·6
1977	1237	167	11·9
1978	1247	125	9·1
1979	966	233	19·4

† The factor 4·8 corresponds to a nitrogen factor of 3·3 compared with, for example, N factors of 3·45 and 3·55 used in the U.K. for pork and beef, respectively. This results in approximately 5 per cent. more lean meat being reported than would be the case in the U.K. The data reported here are thus not directly comparable with U.K. results. (Editor).

TABLE II

AVERAGE MEAT CONTENTS OF SAUSAGES AND SAUSAGE MEAT

	Mean *per cent.*	*s.d.*	Median *per cent.*
1976	78·9	4·31	77·3
1977	79·6	4·60	78·3
1978	80·0	4·36	79·2
1979	79·8	4·81	78·4

TABLE III

AVERAGE FAT CONTENTS OF SAUSAGES AND SAUSAGE MEAT

	Mean *per cent.*	*s.d.*	Median *per cent.*
1976	20·3	4·35	20·7
1977	20·9	4·21	21·4
1978	19·7	3·99	22·6
1979	23·0	3·45	23·7

TABLE IV

PERCENTAGE DISTRIBUTION OF MEAT CONTENTS OF SAUSAGES AND SAUSAGE MEAT SAMPLES

Meat content *per cent.*	1976	1977	1978	1979
75–77·9	53·4	43·5	36·6	43·1
78–80·9	19·4	24·5	24·4	22·7
81–83·9	14·1	15·5	19·4	16·3
84–86·9	9·2	10·7	13·7	11·1
87–89·9	1·7	3·1	3·1	3·4
90–116	2·2	2·7	2·8	3·4

TABLE V

PERCENTAGE DISTRIBUTION OF FAT CONTENTS OF SAUSAGE AND SAUSAGE MEAT SAMPLES

Fat *per cent.*	1976	1977	1978	1979
4–15·9	13·4	11·7	7·2	3·1
16–17·9	9·4	7·4	5·7	4·0
18–19·9	14·5	13·4	10·6	8·2
20–21·9	20·5	17·9	16·8	13·2
22–23·9	17·4	18·8	18·4	19·4
24–25·9	14·0	17·3	19·2	23·7
26–27	10·8	13·5	22·1	28·4

TABLE VI

PERCENTAGE OF SAMPLES NOT COMPLYING WITH LEGISLATION (1976–1979)

Reason for non-compliance	1976	1977	1978	1979
Low meat content	26·0	16·8	22·4	12·0
High fat level	19·1	37·7	44·0	39·9
Excess sulphur dioxide	32·4	27·5	20·8	28·8
Sausage Meat with more than 85 per cent. of meat, containing sulphur dioxide	5·2	7·2	4·8	3·9
Sausage Meat with more than 85 per cent. of meat, containing sulphur dioxide and excess fat	5·8	2·4	4·0	3·9
Added coal-tar colour	0·0	0·6	0·0	1·7
Other*	11·5	7·8	4·0	9·8

* "Other" is made up of combinations of the above reasons for non-compliance. e.g., high fat and sulphur dioxide contents, low meat content and high fat content etc.

TABLE VII

PARTIAL RESULTS FOR SAUSAGES AND SAUSAGE MEAT SAMPLED IN 1975

No. complying	457	
No. not complying	56	(10·9 per cent.)
Mean meat content	79·4	(s.d. 4·32)
Mean fat content	21·5	(s.d. 3·95)
No. of samples with low meat content	7	(12·5 per cent. of non-complying samples)
No. of samples with high fat content	17	(30·4 per cent. of non-complying samples)
No. of samples with excess sulphur dioxide	18	(32·1 per cent. of non-complying samples)

TABLE VIII

COMPARISON OF PERCENTAGES OF ADULTERATION IN VARIOUS MEATS AND MEAT PRODUCTS (1977–1980)

	1977	1978	1979	1980*
Sausages and sausage meat	11·9	9·1	19·4	14·7
Manufactured meat[3]†	7·7	5·0	4·1	6·8
Chopped or minced meats[3]	7·7	6·2	10·0	9·6
Fresh meats[3]‡	0·7	2·2	1·0	0·6

* 1980 results for the period January to May.
† Do not include meat pie adulterations.
‡ Do not include tripe adulterations.

Discussion

Chi squared tests of the data used to compile Table I show that the number of samples submitted between 1976 and 1979 has changed significantly as have the number of non-complying samples. The results from 1979 appear to be the reason for the significant change in the number of non-complying samples. The drop in the number of samples received over the period may be related to price increases for the analyses of sausages and sausage meats.

The mean and median values for the meat contents of sausages and sausage meat show only small variations which are probably of no practical significance, even though the calculated "*t*" scores using 1976 as a base year do indicate statistically significant differences. The fat values showed more variation, with 1979 values indicating an increased amount of fat in sausages and sausage meats. This 1979 result may have arisen because of high meat price rises in this year encouraging an increase in the amount of fat being used. These high price rises may also be reflected in the slight lowering of the average lean meat content of sausages and sausage meat compared to the previous years' average values.

The meat content distribution shows that the majority of sausages and sausage meats have between 75 and 86 per cent. of meat, with most having between 75 and 77 per cent. meat, indicating very good control of the manufacturing process from the producer's view-point. In contrast to this result the fat distribution is spread more evenly over the range of 20 to 27 per cent. There is, however, an apparent trend in the increase of the upper fat levels with 24·8 per cent. of samples having between 24 and 27 per cent. fat in 1976 increasing to 52·1 per cent. for the same range in 1979. This increase is also reflected to some extent in the mean and median fat values for the same period.

Sausages and sausage meat with low meat contents, excess fat and high sulphur dioxide levels are the most common reasons for non-compliance, with high fat values being the most frequently occurring. It is perhaps interesting to conjecture that a low meat content is readily understood by a consumer or magistrate whereas the harm of excess sulphur dioxide or fat is much less apparent. Although it might appear unjust to prohibit sulphur dioxide in sausage meat with more than 85 per cent. meat content the ease with which such a product could be sold as a higher priced minced meat (to which such sausage meat bears an almost identical appearance) does require some control and the sulphur dioxide requirement has been found to be the most satisfactory method so far devised. Coal-tar colours are not permitted in sausage and sausage meat in Victoria and their use is infrequent.

Although complete data for 1975 were not available, those that were showed very similar values to results for the years 1976 to 1979, again indicating a high degree of control by sausage manufacturers.

The results of Table VIII show that the levels of adulteration of sausage and sausage meat are the highest amongst the various types of meat products sampled in Victoria. This position can be taken as an indication of the ease with which sausages and sausage meat are liable to be adulterated compared to fresh cuts of meat, which offer far fewer opportunities for adulteration.

Conclusion

Sausages and sausage meat are the most frequently sampled foodstuffs in Victoria. The majority of samples comply with the statutory requirements, with a persistent proportion failing to comply because of three main reasons, namely, low meat content, excess fat and high sulphur dioxide levels. The level of non-compliance ranged from 9·1 to 19·4 per cent, which when compared with some reported English data[4] indicates that gross adulteration is not occurring. If the results for non-compliance in 1979 are abnormally high (and the preliminary 1980 figures tend to imply this) the average non-compliance for sausages and sausage meat is about 10 per cent. of all such products sold.

References

1. Food and Drug Standards Regulations, 1966, Department of Health, Victoria, Regulation 110.
2. "Official Methods of Analyses of the Association of Official Analytical Chemists", 13th edition, 1980, Washington D.C. 24.006–24.008.
3. Unpublished data collected from samples submitted during the years 1976 to 1980.
4. Annual Report of the County Analyst and Scientific Adviser, Lancashire County Council, 1974, Preston, U.K. p. 48; 1975, p. 10, and 1977, p. 14.

J. Assoc. Publ. Analysts, 1985, **23**, 99–102

Book Reviews

PHYSICO-CHEMICAL BEHAVIOUR OF ATMOSPHERIC POLLUTANTS. Edited by B. Versino and G. Angeletti. D. Reidel Publishing Company, Dordrecht, 1984. Price £59.75. XIV + 666 pp.

This book is an account of the Third European Symposium held in Varese during April 1984.

It is a tribute to the efficiency of the publishers that this volume could be prepared and ready for sale within six months of the symposium, and one at which about 67 papers were given.

These have been collected into five different groups which are all related to the physico-chemistry of air pollutants and includes identification and analysis, characterisation of aerosols, and pollutant cycles, whilst particular emphasis has been put upon the physico chemical aspects of acid deposition.

The symposium was held under the aegis of the E.E.C., who have provided the two Editors. The main language is English, French and German do follow, but each paper has an English summary. The various authors however are multinational. It must be added that all papers have adequate references.

Within the scope of a review of reasonable length, it is not possible to mention all of the papers given, but of particular interest is the development of sampling techniques to evaluate species of major interest in the conversion of pollutants and of their cycle in the atmosphere, as well as new means of investigation for atmospheric pollutants.

Allegrini and others have developed a full methodology for evaluating atmospheric acidity.

Ten Brink of the Netherlands Energy Research Centre, on leave in Brookhaven National Laboratory U.S.A., together with members of the staff of that laboratory investigated measurement of hydrogen peroxide in ambient atmospheres. This peroxide was formed from ozone. Of considerable interest is a paper on the formation and detection of peroxyacetyl nitrate by some German workers who point out that the high concentrations in Southern California smog are rarely approached in Europe.

Workers use sophisticated laboratory techniques and equipment, even airplanes, to monitor various constituents in which interest is shown, but Swedish workers have monitored polycyclic aromatic hydrocarbons (PAH) using elm and kale leaves in various areas, obtaining profiles from different sites with different exposure times, and concluding that the PAH content rapidly decreased with increased distance from busy roads. This may be self evident, but it does need stating, as observation on the plant preceded the work and simple observation does often appear to be an attribute somewhat lacking in some modern scientists. Particle induced X-ray emission (PIXE) is the subject of a

paper by the Director General for Science, Research and Development (I. V. Mitchell) of the C.E.C. who points out that it is valuable as it is non-destructive and also detects many elements simultaneously at the 10–12 ng level.

Considerable progress has been made on investigations into atmospheric transformations since the previous symposium in 1981 especially on nitrogen and sulphur, hydrocarbons, organic halogens and oxygenated hydrocarbons whilst a new topic requiring understanding is the behaviour of atmospheric mercury.

A section deals with aerosols, their measurement and production in different meteorological conditions. Some work done during smog conditions in Berlin suggests that polyaromatic hydrocarbons are associated with particles of diameter less than 2.1 μm, in other words that absorption on Aitken nuclei is important.

A short paper from Yugoslavia deals with suspended particulates in the area of a lead smelter during the winter season. This was selected as the summer in the particular area is always wet and windy, hence results are less reliable. As one would expect lead concentrations in particulate deposits fall off with distance and small particles predominate at the distant site.

It is pleasing that many aerosol measurements have been made using aircraft which obtain samples whilst flying in cloud but do not apparently register the height at which they are flying.

In the past, investigations have often been concerned with point sources of pollution, but a wider view is now being taken especially because of acid deposition and damage to forest and lake and other fresh water systems. Not only is the obvious acid—sulphur dioxide—being investigated, but also NO_2 in its various forms as well as origins. Much energy and expertise is now being devoted to investigations of pollutant cycles in parts of the world previously neglected, and which tend to show that vehicles and their density are now one of the major factors where it would appear from some results that only a small fraction of the pollution moves away from its source.

Studies of snow samples were made in the Antarctic where the predominant role played by atmospheric acids (HCl, HNO_3, and H_2SO_4) is stressed. French workers have also shown that a large algal field in Brittany is responsible—naturally—for generation of dimethyl sulphide and which with favourable winds may be carried across the Atlantic Ocean but of course reacts with other pollutants on its journey.

The importance of correct sampling site and avoidance of contamination during sampling and analysis is stressed in a paper on toxic metals in high altitude glaciers, snow and ice from Grenoble.

In the final summary, the section Chairman, A. J. Elshout of Arnhem, stated that one of the main interests of these investigations is directed to a better understanding of the impact of air pollutants, especially in view of the "acid deposition" problem. Though so much more is known yet it is still not known what causes deposition at a particular location or how the deposition may be influenced by a change in emission. The participants of this symposium are already working towards elucidation of many problems raised, and hopefully many general problems may be solved by the next Symposium.

The book is a worthwhile collection of papers, of considerable interest to environmentalists and, despite the apparent high cost, is well worth having on the library shelf.

G. V. JAMES

PRACTICAL ANALYTICAL ELECTRON MICROSCOPY IN MATERIALS SCIENCE. By David B. Williams. Verlag-chemico International, Deerfield Beach, Florida, 1984. Price $34.95. 146 pp. + 7 pp. index.

The author is in the Department of Metallurgy and Materials Science at Lehigh University, Bethlehem, Pennsylvania, and he has written this short book as it should be of interest to chemists specialising in stereochemical analysis, NMR spectroscopy, organic chemistry, etc.

Dr Williams graduated from Cambridge before eventually becoming Professor and Director of the Electron Optical Laboratory at Lehigh. The short Foreword does point out that the technique of analytical electron microscopy has developed to the stage where it is accurate, reproducible and can be used to solve problems as well as increase our knowledge of the basic structure of materials.

The book is in six chapters, glossy paper, each chapter being well referenced. The introductory chapter deals with metallurgical applications whilst the second chapter concerns alignment and calibration of the analytical electron microscope.

Only a few pages of the book deal with present problems of public analysts, and these deal with polymers, but taking a long term view and considering the title "Scientific Adviser" which many analysts possess, the methods are worthy of consideration for wider work in the future.

The book has many illustrations, is well explained, easily read and certainly should be included in the library of the larger laboratory of the not too distant future.

G. V. JAMES

MAXIMUM CONCENTRATIONS AT THE WORKPLACE AND BIOLOGICAL TOLERANCE Values for Working Materials 1984. Edited by the Commission of the Deutsche Forschungsgemeinschaft for Investigation of Health Hazards of Chemical Compounds in the Work Area. Price DM23. 92 pp. Berlag Chemie, Weinheim, 1984.

This softback would be of wider application and use had it possessed an English summary. It is of interest that the "MAK" values have been used in West Germany as a basis for legal measures, i.e. limits of concentration in working atmospheres and it is claimed that other countries have made use of them.

The book is revised annually by experts from many scientific disciplines and in this list there are about thirty new or altered values, especially for carcinogens and polycyclic aromatic hydrocarbons whilst the values for fine dusts have also been revised.

The limits of the various substances are quoted as p.p.m. (or ml/m^3 and mg/m^3) but the book, which is clearly printed, can only be recommended to German readers.

G. V. JAMES