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Veterinary Residues in Foods and Feeds
Part II
Analysis of Animal Products for Drug Residues

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Problems in the analysis of animal products for residues of medicinal additives are discussed. A survey of current methods for the determination of coccidiostats, anthelmintics, antibiotics, sulphonamides, chloramphenicol, and hormones is presented. Data on residual levels of drugs in animal tissues are surveyed.

In part one of this paper, some current uses of medicinal additives in animal feedingstuffs were described, and some problems, topical interests and concerns resulting from such use were discussed. It was also indicated that the analytical chemist and, in particular enforcement analysts, have a crucial role to play, not only in protecting the public but also in ensuring that the data upon which legal and administrative decisions are based are sound and reliable. The determination of drug residues in animal products provides a good illustration of the difficulties still facing analytical chemists today, even though the equipment available is so much more advanced than in time past. Such difficulties arise from the fact that:

(1) Any residues present are likely to be in the part per billion range, or even lower still in the case of hormones. Hence, very sensitive methods for detection will be required.
(2) Animal products comprise a diverse group of materials differing widely in chemical composition. Thus, techniques with good resolving power will be required to separate the analyte from other compounds co-extracted from the matrix.
(3) The residues of interest differ widely in molecular structure and chemical and physical properties. This increases the difficulty of devising a method which can be utilised for the detection and determination of a wide range of residues in a single analysis. Additionally, it may be necessary to discriminate between two complex molecules of very similar chemical structure, e.g. salinomycin and narasin. (Fig. 1).

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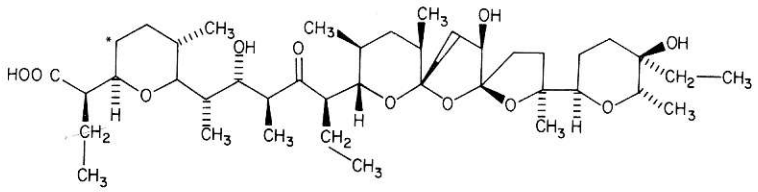

Fig. 1. (a) Salinomycin, $C_{42}H_{70}O_{11}$. (b) Narasin, $C_{43}H_{72}O_{11}$.

(4) The need to survey large numbers of samples demands methods that are rapid, capable of automation, cheap and simple to operate. This requirement is in direct opposition to the limitations imposed in 1 to 3 above. Hence, the usual compromise is to employ rapid screening methods for a wide range of samples. Where apparent positives are detected, those samples only are subjected to a more selective analytical technique, e.g. mass spectrometry, to confirm the initial findings.

The wide range of analytical techniques available to the residue chemist, e.g. capillary and packed-column GLC in conjunction with several selective detectors, HPLC in a variety of separation modes, fluorimetry, mass spectrometry, RIA and more recently, other immunochemical approaches such as ELISA, contrast with the more direct approach taken to solve analytical problems in years past.

Until about 30 years ago, the main problems faced by analytical chemists were: (1) getting the sample into solution; (2) removing interferents; (3) determination of the analyte by titrimetry, gravimetry or colorimetric analysis.

Even though considerable manipulative and technical skill was required in those days (possibly more so than with today's microprocessor controlled black boxes!) classical analytical techniques cannot solve the problems posed by the increasing sophistication of modern processing technology, particularly in the food and agriculture industries where analytical chemists are frequently asked to measure minute levels of organic molecules in a complex organic matrix. Analytical chemists have now become problem solvers as well as analysts and as such must contribute to the whole process as well as the determination itself. This is illustrated below:

The analytical process

(1) Definition of the problem.
(2) Sampling.
(3) Extraction and Separation.
(4) Measurement.
(5) Data processing.
(6) Solution of the problem.

Some of the problems resulting from the use of residues of medicinal additives in feeds and animal tissues have been discussed in Part I of this paper. Aspects of more direct relevance to the analyst, particularly to the enforcement analyst,

will now be described. A short account is given of the methods available for the determination of coccidiostats, anthelmintics, antibiotics and hormones in animal tissues such as muscle, fat, offal, milk and eggs.

Coccidiostats

The development of analytical methods for the determination of residues of coccidiostats in animal products has still a long way to go before satisfactory methods are available. Normally the approach used is to investigate the corresponding method for the determination of the coccidiostat in an animal feed and then attempt to improve the sensitivity and limit of detection to the point where it can be used to detect and determine the much lower (residual) levels found in animal tissues. Hence, some of the current developments in methodology for the determination of coccidiostats in feeds will first be reviewed.

Most analytical methods for this purpose comprise a simple solvent extraction followed by a clean-up on a conventional chromatographic column and measurement by spectrophotometry. Whilst individual methods operate down to a stated limit of detection, which is satisfactory bearing in mind the normal level of incorporation of the drug into the feed, existing methods are not always specific to the named compound and interferences can occur even at the relatively high levels occurring in feeds. With the advent of HPLC, much work is now in progress to develop methods using this technique in the hope that they will prove quicker, more sensitive and less subject to interference from other substances present in the feed. In the last few years the Analytical Methods Committee of the Royal Society of Chemistry has published new and recommended methods for halofuginone[1], nifursol[2], olaquindox[3] and ronidazole[4]—all based on HPLC, whilst GLC was recommended for pyrimethamine[5]. However, most medicinal additives are non-volatile and, hence, cannot be determined by GLC without prior derivatisation. HPLC methods are currently being developed for arprinocid, lasalocid and sulphadimidine. The Committee of Experts of the EEC has also been encouraging the change from colorimetric methods to procedures based on HPLC. However, this committee has now not met for two years and although several new methods have been approved, they have not yet been promulgated into the legislation. In addition, there are a number of papers published by individual groups of research workers from industrial and governmental laboratories describing the use of HPLC for the determination of certain coccidiostats in feeds. However, such methods have not yet been evaluated in other laboratories or subjected to collaborative study.

Problems encountered in the analysis of animal products for coccidiostats today are in many ways similar to those with which analysts had to struggle in the early days of pesticide residue determinations in the 1960s. Levels are very low, the chemical present is in general not known to the analyst and methods are required for a number of compounds which differ markedly in their chemical characteristics and properties. Analytical methods in such circumstances are generally time consuming and expensive and may require the use of confirmatory techniques such as mass spectrometry. In the early days, methods of analysis were developed for individual insecticides. Later work was devoted to

the so-called multi-residue methods by which a number of compounds could be identified and measured in one single analysis, e.g. organochlorines and herbicides, thus maximising the information obtained with minimal additional cost. Accordingly, chemists at the Laboratory of the Government Chemist (LGC) have been endeavouring to develop multi-residue methods for drugs in animal feeds with the ultimate objective of applying similar systems for the determination of residues in animal products. Some of their latest work is summarised below.

Identification of drugs in feeds

In developing a new analytical method it is usual to work in reverse order, i.e. to investigate the end determination stage first. Once this has been completed, it can be used in investigations on the extraction and clean-up stages of the analytical procedure. At the LGC, a reverse-phase HPLC separation and detection system has been used for the identification of medicinal additives extracted from feeds. Obviously it is not possible to separate all permitted compounds isocratically on a single chromatogram. Rather than use gradient elution systems we have opted for a single column with a series of mobile phases consisting of a pH buffer containing increasing proportions of acetonitrile to elute all common medicinal additives from the column in a predictable order. An example of the separation achieved is shown in Figure 2. Most combinations

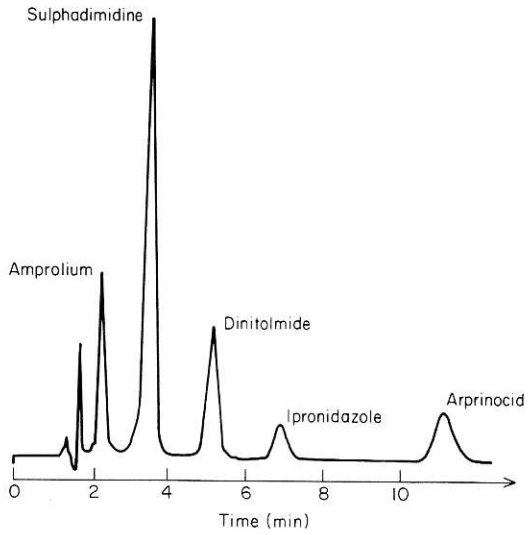

Fig. 2. Drug separation scheme. HPLC (reverse-phase) separation/identification of medicinal additives in feeds, at wavelength 254 nm. Mobile phase MeCN/H_2O (22·5/77·5).

of drugs can thus be separated and a presumptive identification made by injection onto the column and selection of the appropriate mobile phase. Even so, there are occasions where it would not be possible to separate certain pairs of drugs and this could lead to an incorrect identification. In such cases, it may prove possible to resolve the problem by an appropriate choice of wavelength

for measurement, e.g. 254 or 310 nm. Different compounds may exhibit significantly different molar extinction coefficients at different wavelengths. This is shown in Figures 3 and 4. Note, at $\lambda = 254$ nm, the strong peak for amprolium and dinitolmide but weak for ipronidazole, whereas at 310 nm there is a weak response for amprolium and dinitolmide but strong for ipronidazole. Hence the detection wavelength can be used diagnostically.

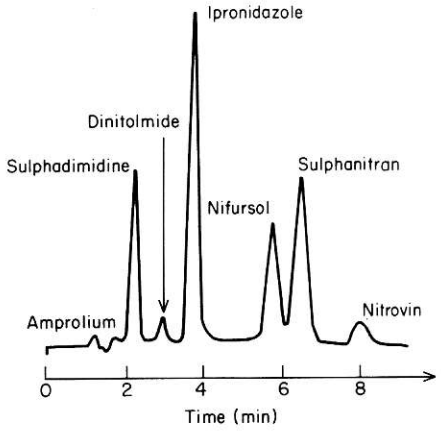

Fig. 3. Drug separation scheme. HPLC separation/identification of medicinal additives in feeds at wavelength 310 nm. Mobile phase MeCN/H_2O (35/65).

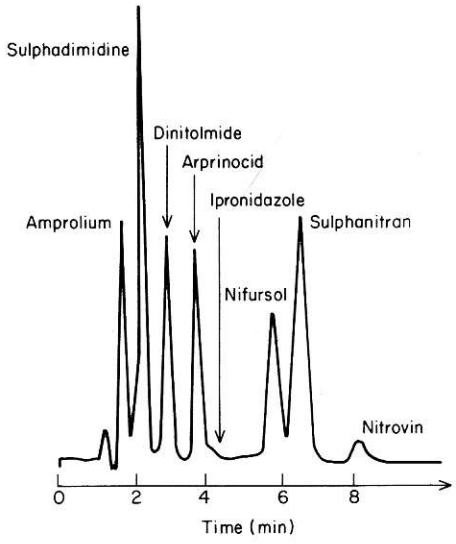

Fig. 4. Drug separation scheme. HPLC separation/identification of medicinal additives in feeds at wavelength 254 nm. Mobile phase MeCN/H_2O (35/65).

Extraction from the feed

A number of hydrocarbon, chlorinated and more polar solvents were studied systematically with a view to selecting one, two or possibly three solvents for extraction. Obviously the solvent must be a good solvent for the drugs under investigation but as poor a solvent as possible for other constituents, to reduce the amount of co-extractants and the degree of clean-up required prior to injection on to the HPLC column. The solubility of the drugs in each solvent was first tested. No temperature control was included in these experiments and there was no guarantee that equilibration had been attained. Nevertheless, the study did provide a quantitative indication of the relative solubility of each drug in each of the solvents tested. Most drugs were found to be soluble in solvents such as chloroform, dichloromethane (DCM), acetonitrile, dimethylformamide or methanol, using concentrations of the same order as would be encountered in a typical extraction. Furthermore, most drugs are insoluble in hexane. Hence, it may well prove possible to make use of this property and to pre-extract a sample with hexane to remove fat from the feed which might interfere at later stages of the determination. At the same time this would lessen the degree of clean-up required to remove interferences and protect the HPLC columns from deterioration. The efficacy of selected solvents was then tested by preparing a premix of the drug at a level of 0·5 per cent. or 1 per cent. in an inert carrier ($CaCO_3$). The premixes were stored in amber jars to reduce photodegradation and then added to six different animal feeds: Pig feed, grower and starter; Turkey, grower and starter; Broiler, starter and finisher. These fortified feeds were then ground in a pestle and mortar and tumbled to give a homogeneous mix. The levels of fortification were selected to be close to normal commercial use and the feeds varied in proximate analysis, content of fishmeal etc. Early results have shown that DCM is less suitable as an extraction solvent than acetonitrile as too much co-extracted material is present. Clean-up using Sep-Pak cartridges is then required. Most drugs can readily be eluted from Sep-Paks using methanol/water. In some cases, recoveries are nearly quantitative.

It is hoped that this work will lead eventually to the development of a multi-residue approach for coccidiostats in animal products.

Animal Products

The AMC Veterinary Residues Sub-Committee is currently developing validated methods for the determination of drug residues in animal tissue. Methods are required which can be used quantitatively down to 0·1 mg/kg and lower if possible. A procedure for the determination of halofuginone in chicken tissues has been published[6]. After digestion with trypsin, drug residues are extracted with ethyl acetate, partitioned into an aqueous buffer, passed through a Sep-Pak C_{18} cartridge and then examined by HPLC using a reverse-phase column and UV detector. In this case it was possible to check the procedure using a ^{14}C-labelled compound fed to the chickens. A method for dimetridazole has also been published[7].

One of the major difficulties in this type of work is the provision of material containing suitable and known amounts of test substance to validate the analytical procedure. Most drugs are metabolised fairly rapidly leading to low levels in the tissues, especially where no conjugation takes place. In the case of dimetridazole, it was necessary to feed the chickens elevated levels of the drug to produce measureable levels in the tissues even with a zero withdrawal period. However, in this type of work, "spiking", i.e. addition of known amounts immediately prior to analysis is not thought to be very satisfactory.

Other methods in the literature have been proposed for amprolium[8], clopidol[9,10] and sulphaquinoxaline[11], but currently we are a long way from the multi-residue approach which is needed.

Anthelmintics (Anti-worm preparations)

The review[12] by Watson was noted in Part I of this paper. A quick summary of the present position is as follows.

FENBENDAZOLE

This is used typically at a dose level of 5–10 mg/kg body weight. Such a dose will give rise to residues in the range 0·7 to 1·6 μg/ml (p.p.m.) in serum and 0·3 μg/ml in milk after 1–2 days. The liver is the target organ, where 7·9 mg/kg has been detected after 2 days, reducing to below 0·1 mg/kg after 14 days, the recommended withdrawal time.

OXFENDAZOLE

A 3 mg/kg body weight oral dose peaks at 0·5 p.p.m. in plasma after 6–9 h, then declines to <0·1 p.p.m. after 48 h.

LEVAMISOLE

A paper in Food Additives and Contaminants by Österdahl *et al.*[13] describes some experiments with cows suffering from lungworms. They were given a dose of 7 mg/kg body weight between the morning and afternoon milking. The highest residue levels (2·5 μg/ml) in the milk were found 1 h after administration of the drug. Levamisole is metabolised rapidly with half life of about 5 h. The recommended withdrawal time of two days is, therefore, satisfactory. The analytical method used was precipitation of proteinaceous matter from the milk, extraction, and clean-up on an Extrelut column. Measurements were made on the eluate from a reverse-phase HPLC by a UV detector. The limit of detection was 0·04 μg/ml and at 0·3 μg/ml recoveries were 71–91 per cent.

Antibiotics

The main types of antibiotics used in animal production can be classified as follows: (i) β-lactam compounds, e.g. the penicillins; (ii) sulphonamides, e.g. sulphadimidine; (iii) tetracyclines; (iv) ionophores; (v) other compounds, some with multifunctional action, e.g. coccidiostats, antifungals, aminoglycosides.

Because of the wide diversity of compounds encountered and the large number of tests involved in a survey of fresh meat products for antibiotic

residues at slaughterhouses in the U.K., screening methods are generally employed in the first instance. Presumptive evidence of the presence of an active compound can then be confirmed by additional, more expensive techniques, but on a much more restricted number of samples.

The method proposed for use within the EEC has been designated the Four Plate Test (FPT) or Frontier Post Test or indeed False Positive Test, since under certain conditions it can give rise to false positive results, especially with pig offal and meat which has undergone microbial spoilage. The test described by Bogaerts and Wolf[14] is reasonably sensitive to most antibiotics (Table I) but gives little information as to the identity of the substance present. In the test, the samples are applied directly to the surface of four plates of agar media inoculated with *B. subtilis* spores at pH 6, 7·2 and 8 with the fourth plate containing *Micrococcus luteus* at pH 8. Trimethoprim is added to the pH 7·2 medium to enhance the sensitivity of the test to sulphonamides. Diffusion of an antibacterial substance is indicated by the formation of a zone of inhibition on the plate after incubation.

As can be seen in Table I, the test is very sensitive to the penicillins and tetracyclines but of little use for the detection of chloramphenicol residues. Nevertheless, the test has its uses, although some workers have suggested that not all four plates are really necessary. Some interfering substances can be removed by TLC (Smither[15]). Offal and contaminated samples are most likely to contain naturally occurring inhibitory substances, presumably derived from earlier bacterial activity within the original sample. However, the chief drawback of the FPT test is that it is only capable of detecting inhibitory activity without identifying the compound responsible.

Electrophoresis is now used as a confirmatory procedure. The technique has been described by Smither and Vaughan[16]. They used high voltage equipment to measure migration distances of 50 antibacterial agents in agar and agarose gels at both pH 6 and pH 8. Comparison of the different migration distances in the two gels formed the basis for the identification. Bio-autography, using *B.cereus* var. *mycoides* or *Micrococcus luteus*, was used for visualisation. No trouble from naturally occurring inhibitors was encountered and significantly better detection limits were claimed (Table I) except for sulphonamides.

TABLE I

DETECTION LIMITS FOR ANTIBIOTICS

Compound	Four Plate Test μg/g	Electrophoresis μg
Avoparcin	7	0·1
Bacitracin	1·0	0·05
Chloramphenicol	10	0·5
Monensin	14	0·5
Penicillin	0·02	0·002
Streptomycin	0·5	0·02
Sulphadimidine	7 (0·5)*	15
Tetracycline	0·1–0·4	0·1

* In presence of trimethoprim.

These tests were used by Smither *et al.*[17] in a survey of U.K. meat for antibiotic residues in samples collected between 1977 and 1979. Of more than 5000 home-produced meat samples examined, only 0·6 per cent. showed inhibitory activity in the FP Test. Further work using electrophoretic techniques showed that only 2 of the 34 failures were due to true antibacterial residues. Of more than 900 samples of imported meat, 8·7 per cent. also showed inhibitory activity. All of these results were subsequently shown to be false positives.

In the U.S.A., sulphonamide residues are frequently detected in pig tissues (14 per cent. violations), other antibiotics being found in less than 1 per cent. of animals tested.

SULPHONAMIDES

Colorimetric and chromatographic methods (TLC, GLC, HPLC) have been described for the detection of sulphonamide residues in meat, fish and milk. Parks[18] examined pig livers for two sulphonamides at the 100 μg/g level and obtained recoveries of 40–50 per cent. Thomas *et al.*[19] found levels of 60–70 ng/g with an average recovery of 95 per cent. Using HPTLC, levels of sulphamethazine in skim milk or trout can be detected down to 5 ng/g. The limit of detection is less good with whole milk and recoveries are low.

GLC and HPLC techniques have also been employed. Extensive clean-up is required and the methods are not suitable for screening purposes. GC-MS with single ion monitoring has been used for confirmation of identity. Typically, for GLC evaluation, sulphonamide residues are extracted with acetone and acidified. After removal of acetone the aqueous fraction is partitioned with dichloromethane (DCM), then made neutral and the sulphonamides then extracted with DCM. The sulphonamides are then methylated with diazo methane in ether, separated on a 25 m × 0·25 mm WCOT glass column coated with OV7, or equivalent, and detected by electron capture. Obviously, this is a long and tedious procedure not suitable for screening purposes. However, it enables a large number of different sulphonamide durgs to be detected and identified.

CHLORAMPHENICOL

Chloramphenicol (CAP) is a powerful broad-spectrum antibiotic which acts against Gram-positive and Gram-negative micro-organisms. However, it does have a number of undesirable side effects including toxicity to bone marrow, resulting in severe, irreversible and often fatal aplastic anaemia. There is some evidence of illegal use of the drug in animal husbandry and concern that residues might find their way into human food.

In addition to microbiological methods, a number of chemical techniques have been described for the determination of CAP residues in animal tissues. Thus GLC, following derivatisation to form trimethylsilyl ethers or heptafluorobutyric anhydrides with electron-capture detection, or HPLC with a UV detector, or colorimetric methods have all been proposed. Colorimetry is not sensitive (~10 mg/l in milk) whereas HPLC will determine down to about 0·1 mg/l and GLC to about 0·01 mg/l.

At the LGC, the use of immunoassay techniques and their application to "difficult" areas of analytical chemistry has been investigated, particularly for food and veterinary residue determinations. In conjunction with St Bartholomew's Hospital, a number of antibodies against CAP have been prepared and are currently being evaluated for use in an ELISA method. An American publication suggests that ELISA methods for chloramphenicol may have a sensitivity approaching 1 μg/kg. Initial findings are very encouraging. Antiserum titre curves using a ^{14}C labelled CAP have been produced, and a satisfactory displacement observed.

Hormones

Radioimmunoassay (RIA) methods have been developed for the detection and determination of residues of the synthetic anabolic agents diethylstilboestrol, hexoestrol, zeranol, trenbolone (and its main metabolite 17α-hydroxytrenbolone) in animal fluids (urine, bile) and tissues. Most of this work was carried out at the Institute for Research on Animal Diseases (Agriculture and Food Research Council), Compton, under the supervision of Dr. R. Heitzman, but sadly this Section of the Institute has recently been closed down. RIA methods are, of course, very sensitive, and amenable to automation in many cases, but they do require expensive facilities and equipment which is not generally available even in many modern and well-financed analytical laboratories. Furthermore, the method requires a suitable antiserum as well as a labelled form of the analyte. Nowadays, labelled compounds are available commercially. Obtaining the antiserum is more difficult (although some antisera are now available commercially), and it may vary from batch to batch. Cross reactivity may be a problem and at the very low levels of measurement (picograms) adsorption on to glass surfaces can cause difficulties. Some active compounds conjugate to proteins and may need to be released with a suitable enzyme. Similar studies are proceeding at MAFF Norwich and the Central Veterinary Laboratory, Weybridge. Figure 5 illustrates a typical curve obtained and the

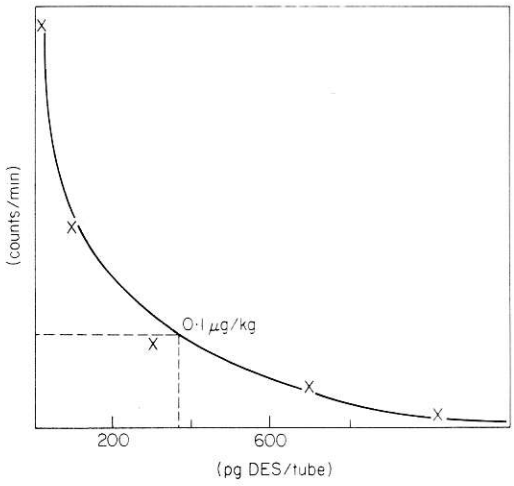

Fig. 5.

extreme sensitivity which can be achieved. Residue levels of anabolic hormones in meat after different forms of treatment are illustrated in Table II. This shows that dangerous levels will only occur at the site of implant or injection and provided that an adequate waiting period is observed following treatment, it is not possible to distinguish reliably between treated and untreated animals. The maximum levels of anabolic steroids derived from the meat of treated animals are shown in Table III by comparison with the naturally occurring levels of hormones. Hence, the treatment of animals with natural steroids results in residues in meat that are several orders of magnitude lower than those that occur naturally in bulls or pregnant cows.

TABLE II

RESIDUES OF ANABOLIC AGENTS IN CATTLE TISSUES (pg/g) (WHO[20])

Compound determined	Animal	Tissue examined			
		Muscle	Liver	Kidney	Fat
Testosterone	bull	535	749	2783	10950
	heifer	92	193	595	250
	veal calf	16	39	256	685
	treated[a]	70	47	685	340
Trenbolone	steer[b]	50	230	50	80
	steer[c]	50	50	20	80
	veal calf[d]	127	521	235	388
	veal calf[e]	797	3467	2563	2580
	veal calf[f]	1673	4930	4083	8893
DES	veal calf[g]	90	270	770	n.d.
	veal calf[h]	540	18900	8900	8300
	veal calf[i]	120–210	2300	1500	—
	veal calf[j]	50	240	170	—

[a] Slaughtered 77 days after implantation of 20 mg oestradiol-17β + 200 mg testosterone.
[b] Slaughtered 77 days after implantation of 40 mg oestradiol-17β + 200 mg TBA.
[c] Implant of 40 mg oestradiol-17β + 200 mg TBA, implant removed after 60 days, slaughtered after 15 more days.
[d] Slaughtered 77 days after implantation of 20 mg oestradiol-17β + 140 mg TBA.
[e] Slaughtered 77 days after implantation of 200 mg oestradiol-17β + 1400 mg TBA.
[f] Slaughtered 77 days after implantation of 500 mg oestradiol-17β + 3500 mg TBA.
[g] Slaughtered 4 days after intramuscular injection of 100 mg DES.
[h] Slaughtered 7 days after intramuscular injection of 200 mg DES—isopropionate.
[i] Slaughtered 28 days after intramuscular injection of 150 mg DES—isopropionate.
[j] Slaughtered 90 days after intramuscular injection of 150 mg DES—isopropionate.

TABLE III

INTAKE OF ANABOLIC STEROIDS FROM MEAT (WHO[20])

	Testosterone	Oestrogen	Progesterone
Production in humans (μg/day)			
Adult male	6480	136	416
Adult female	140–320	46–64300	326–29400
Maximum amounts of hormone (μg) in 250 g meat			
Untreated cattle	0·13	0·11	2·5
Treated steer	0·0006	0·005	0·15
Treated heifer	0·025	0·005	—

Conclusions

There is no evidence that the use of medicinal additives in animal feedingstuffs constitutes any danger to the animals themselves or to humans consuming edible animal products, providing only that the conditions of use are strictly adhered to. Nevertheless, there is a need for better analytical procedures so that the situation can be monitored continually, to provide reassurance that all necessary precautions are being observed.

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The Connective Tissue Content of Male Forequarter Bovine Cuts

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Data on the composition, particularly the connective tissue content of trimmed bovine brisket, chuck and blade, clod and neck 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 co-operated in an earlier survey[1] of bovine cuts (skin, flank, diaphragm and masseter muscles).

The same group has now completed a further survey of four other bovine cuts viz; brisket; clod; chuck and blade; and neck muscle. Compositional analysis was undertaken in the laboratories of the Greater Manchester County Analyst, the Lancashire County Analyst (the co-ordinating laboratory), Merseyside County Analyst and of Messrs. Ruddock & Sherratt. The four cuts are parts of the forequarter of the animal and are known to be used in the manufacture of meat products. This survey concentrated on cuts of bullock origin and was particularly aimed at establishing data on the connective tissue content of the lean part of the cuts although other useful data are provided.

Connective tissue is defined as in a previous paper[2], i.e.

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

Sampling and Sub-sampling

All samples of the four cuts were taken from male bovine animals up to 18 months old. 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.

Approximately 5 lb samples of the four cuts 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 had previously 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 fifteen samples of the bovine cuts from home produced bullocks were analysed.

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Methods of Analysis and Quality Assurance

Each laboratory used its normal operational methods for determination of water, ash, fat (acid digestion procedures), 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[3].

During the survey, each laboratory was required to adopt a minimum within-laboratory quality control rate of 20 per cent., i.e. one in five determinations were to be replicated, twice the minimum rate recommended by the Association of Public Analysts[4]. Laboratories were advised to continue their normal recovery quality control procedures for nitrogen determination (dl-Alanine and nicotinic acid are used by some laboratories for internal quality control). In addition each laboratory was required to analyse a Community Bureau of Reference material[5], namely BCR No. 63, at a rate equivalent to not less than three nitrogen determinations on the reference material for every ten nitrogen determinations on the meat cut. The results are shown in Table I. Each

TABLE I

NITROGEN CONTENTS OF STANDARD REFERENCE MATERIAL* IN COLLABORATIVE SURVEY

	Lab 1	Lab 2	Lab 3	Lab 4
Mean nitrogen content found (per cent.)	5·80	5·82	5·90	5·82
Intra-laboratory† standard deviation	0·05	0·05	0·05	0·02
Difference from overall mean as percentage of overall mean	−0·7	−0·3	+1·0	−0·3

* BCR No. 63 (Community Bureau of Reference)—Skim Milk Powder Performance specification (containing 95 per cent. of the population of the laboratory means) = Certified Value ± 2 standard deviations (5·83 ± 0·092 Range 5·74–5·92).

† Replication was carried out by two analysts over two circulations.

laboratory's results fell within BCR specification. The acceptability of other internal quality control data was assessed by individual laboratories by their usual procedures. The programme of within laboratory quality assurance was supplemented by a small programme of between-laboratory quality assurance.

Two limited collaborative trials were carried out to assist in monitoring the quality of analytical data, namely, on two occasions four meat based materials were circulated amongst the four laboratories for hydroxyproline determination. The results from these trials are shown in Table II. The absolute reproducibilities obtained fell within the expected range at each hydroxyproline level[6].

Table III summarises the analytical data for each of the bovine cuts. Wet-fat-free-connective tissue content has been calculated as indicated earlier and connective tissue levels for each group of samples are shown.

TABLE II

HYDROXYPROLINE DETERMINED ON FOUR CIRCULATED MATERIALS

	Mean hydroxyproline content *per cent.**			
	Lab 1	Lab 2	Lab 3	Lab 4
Sample 1	0·24	0·24	0·26	0·24
Sample 2	0·66	0·64	0·70	0·66
Sample 3	0·10	0·10	0·12	0·10
Sample 4	0·65	0·63	0·66	0·61

* Each the mean of the results obtained by two analysts.

Discussion

The trimming of the cuts of brisket; clod; chuck and blade; and neck 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. It is suspected that the cautious trimming procedure may have left excess fat on certain of the trimmed sub-samples, particularly in the case of brisket. 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 the four cuts would have connective tissue contents (as defined) on average of around 10 per cent. Furthermore none of the 115 samples of the cuts had a connective tissue content above 20 per cent.

The average of about 10 per cent. of connective tissue is consistent with the data for shin, diaphragm, and flank, from the first survey (reproduced as Table IV for comparison). However, the conclusion can be drawn that masseter muscles have a considerably higher connective tissue content than the other seven cuts included in the two surveys with a mean of 19·2 per cent. (range 7·2 to 28·5 per cent.). The earlier recommendation of a 10 to 20 per cent. allowance for the connective tissue content of beefburgers[2] 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 mean for each cut was found to be around 3·6 per cent. This is consistent with similar data from the first survey (Table IV) and not inconsistent with the Analytical Methods Committee recommendation for an average nitrogen factor of 3·55 "as the best compromise for general use"[7].

The Bacon and Meat Manufacturers' Association (BMMA) recognising the importance of the amount of skin, rind, gristle, sinew (connective tissue) which may contribute to the total meat content of a product has produced an advice note[8] for its members. The advice note indicates the BMMA view of the natural connective tissue content of various cuts in terms of collagen content and collagen to total meat protein ratio. Collagen (or collagenous connective tissue) is defined by the BMMA as hydroxyproline per cent. × 8. The collagen/protein ratio is the ratio of collagen as defined to the total meat protein expressed on the dry matter.

TABLE III

THE COMPOSITION OF FOREQUARTER 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.*
Brisket	32	Range	57·1–74·7	2·8–21·5	2·81–3·64	0·11–0·53	3·9–19·5	3·33–4·15
		Mean	68·3	10·9	3·26	0·25	9·1	3·67
		Standard deviation	5·1	6·3	0·24	0·08	3·1	0·19
Chuck and blade	30	Range	65·7–75·6	1·6–13·5	3·12–3·63	0·14–0·39	5·1–14·5	3·38–3·87
		Mean	71·6	6·6	3·34	0·24	8·9	3·58
		Standard deviation	2·6	3·1	0·14	0·08	2·8	0·15
Clod	27	Range	67·8–75·6	1·2–10·6	3·24–3·78	0·1–0·45	3·7–16·7	3·37–3·88
		Mean	73·5	4·8	3·44	0·27	9·8	3·61
		Standard deviation	2·2	2·7	0·15	0·09	3·4	0·14
Neck	26	Range	68·5–75·7	2·7–10·6	3·23–3·66	0·2–0·49	7·5–18·1	3·43–3·83
		Mean	72·4	5·8	3·44	0·34	12·6	3·66
		Standard deviation	2·1	2·6	0·13	0·07	2·7	0·12

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

TABLE IV

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	66·0–76·2	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	1·0–3·7	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	0·10–0·45	3·7–16·7	3·19–4·30
		Mean	69·0	10·5	3·15	0·22	8·2	3·52
		Standard deviation	3·6	4·3	0·22	0·10	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	7·0	3·49	0·25	9·2	3·75
		Standard deviation	3·2	4·0	0·25	0·08	2·8	0·24

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

TABLE V

THE COLLAGEN CONTENT OF EIGHT BOVINE CUTS

Cut	Number of samples		Collagen* *per cent.*	Collagen/protein† *per cent.*
Masseter	27	Range	1·6–6·2	7·8–30·8
		Mean	4·2	19·9
		Standard deviation	1·2	5·8
Shin	26	Range	1·4–3·6	6·4–16·1
		Mean	2·5	11·5
		Standard deviation	0·6	2·7
Diaphragm	27	Range	0·8–3·6	4·3–18·1
		Mean	1·8	9·2
		Standard deviation	0·8	4·0
Flank	27	Range	0·7–3·8	3·3–17·3
		Mean	2	9·1
		Standard deviation	0·6	2·6
Brisket	32	Range	0·8–4·2	3·8–21·1
		Mean	2	9·7
		Standard deviation	0·7	3·6
Chuck and blade	30	Range	1·1–3	5·4–14·8
		Mean	1·9	9·2
		Standard deviation	0·6	2·8
Clod	27	Range	0·8–3·6	3·8–16·4
		Mean	2·1	9·9
		Standard deviation	0·7	3·4
Neck	26	Range	1·6–3·9	7·8–19·1
		Mean	2·7	12·7
		Standard deviation	0·6	2·9

* Collagen = hydroxyproline (per cent.) × 8.
† Collagen/protein = collagen (per cent.)/nitrogen (per cent.) × 6·25 expressed as a percentage.

TABLE VI

COMPARISON OF WET-FAT-FREE-CONNECTIVE TISSUE AND COLLAGEN/PROTEIN RATIO

Cut	Fat *per cent.* (range)	Wet-fat-free *per cent.* (range)	Collagen/protein *per cent.* (range)
Shin	1·0–3·7	6·3–16·7	6·4–16·1
Clod	1·2–10·6	3·7–16·7	3·8–16·4
Neck	2·7–10·6	7·5–18·1	7·8–19·1
Chuck and blade	1·6–13·5	5·1–14·5	5·4–14·8
Masseter	2·4–14·9	7·4–28·5	7·8–30·8
Flank	1·2–20·4	3·3–17·8	3·3–17·3
Diaphragm	4·6–20·9	3·7–16·7	4·3–18·1
Brisket	2·8–21·5	3·9–19·5	3·8–21·1

The data from our survey and its earlier counterpart have been converted into collagen and collagen/protein ratios as defined by the BMMA and these are shown in Table V.

Table VI shows comparative values for the eight bovine cuts viz: connective tissue (wet, fat-free) as defined in this survey (hydroxyproline × 37) and connective tissue as defined by BMMA. Both methods of calculation give a similar result at lower fat and hydroxyproline levels. At the higher levels of either of these two parameters the wet-fat-free-connective tissue expression is slightly lower than the collagenous connective tissue expression. This is particularly noted in the cases of masseter muscles, diaphragm and brisket.

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Nitrogen Content of Seven British Commercial Species of Fish

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Nitrogen contents of five species of fish have been measured over a two year period, two further species were studied over a one year period and the effects of fishing ground and size of fish were examined. All species showed some seasonal variation while fishing ground and size of fish had no discernible effect. The results for nitrogen content are compared with published data.

In the U.K., statutory compositional requirements on fish content apply at present to fishcakes[1] and to certain spreadable fish products[2]; other fish products must meet the general requirements of the Food Act[3], though some may become subject to specific standards in the foreseeable future. Estimation of fish content of fish products is based on measurement of the nitrogen content of the product, suitably corrected for non-fish nitrogen by the Stubbs & More[4] procedure. The nitrogen content of any species of fish, meat or poultry varies somewhat, and it is customary to use an agreed mean nitrogen content usually called the "nitrogen factor". In the case of fish, nitrogen factors have been agreed only for cod[5] and saithe, or coalfish[6]. These factors were derived from a survey of the composition, in the case of cod, of 295 fish caught over a 20 month period, and, in the case of saithe, of 95 fish caught over 23 months but covering only eight months of the year. The derived factors were 2·85 and 2·90 respectively, and are now used by Public Analysts and by industry.

The Analytical Methods Committee did not organise any further surveys but subsequently prepared a list of nitrogen contents for other species of fish based on published information[7].

At the request of the U.K. Association of Frozen Food Producers, Torry Research Station has measured the nitrogen content of five white fish species, haddock, whiting, saithe, ling and North Atlantic hake, over a period of two years from November 1979 to October 1981. A marked seasonal variation in the nitrogen content of haddock and whiting was observed: the cod survey by the Analytical Methods Committee did not clearly indicate a seasonal variation in nitrogen content. For this reason, a further survey of the composition of cod was carried out during 1984. Plaice was studied at the same time. The results of both surveys are given here; analyses were carried out at the same time for moisture, fat and ash contents and for other parameters, and the results will be submitted for publication separately.

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Procedure

The nitrogen content of fish could be expected to vary with the season, with the fishing ground, with the size of the fish and with the treatment of the fish after catching. It proved impossible to survey systematically all these factors. In practice samples were obtained from most grounds accessible to U.K. fishing boats as often as possible. No attempt was made to weight the sample collection according to the yield of any particular ground. Fish were analysed usually after not more than 48 h in ice, though there were a few exceptions; some fish were frozen soon after capture. Fish of different sizes were sought but often the range of sizes obtained was not as great as desired.

Fishing Ground

Haddock, whiting, saithe, ling and North Atlantic hake were obtained from 12 grounds: Shetland, Faroes, Scottish West Coast, Dogger Bank, Aberdeen Bank, Moray Firth, Minches, Outer Hebrides, Norway coast, Orkney, Rockall and German Bight.

Cod and plaice were obtained from five grounds: Shetland, Scottish West Coast, Aberdeen Bank, Moray Firth and Norway coast.

Fish were obtained from a variety of sources, such as the research vessels of Torry Research Station and the Marine Laboratories in Aberdeen and Lowestoft, but mainly from commercial boats landing at Aberdeen Fish Market.

Season

Some grounds, Aberdeen Bank and Shetland, for example, were sampled throughout the year. Other grounds were sampled less frequently, Rockall twice only, for example. During the second year of the 1979/81 survey, gaps due to holidays and bad weather were filled, where possible.

Size

Four fish of the same species were obtained at each sampling. It was hoped that two fish of two substantially different sizes would be included. This was seldom the case, so the length and weight of each fish were measured.

Analytical Methods

Skinned fillets of each fish were minced together and thoroughly mixed. In the earlier survey nitrogen was determined as recommended by the Analytical Methods Committee[8], with one slight variation; aliquots of the acid digest were distilled in duplicate, instead of distilling the total digest. In the second survey, of cod and plaice, a Tecator block digestion unit and Tecator Kjeltec Auto 1130 distillation unit were used. Two grams of sample were digested with 15 ml of sulphuric acid, 10 g of potassium sulphate and 0·2 g of copper(II) sulphate. The total digest was distilled.

In both surveys duplicate determinations were carried out on the prepared sample.

Analysis of Data

Since the various factors to be examined could not be varied systematically, a complete analysis of variance was not possible. The effects of the factors were determined by simple significance tests on the complete set of data or on suitable subsets of the data.

Results and Discussion

The mean nitrogen contents of the seven species of fish are given in Table I, together with standard deviations and ranges.

TABLE I

NITROGEN CONTENTS OF FISH

	Mean *g/100 g*	Standard deviation	Maximum value *g/100 g*	Minimum value *g/100 g*	Number of fish
Cod	2·906	0·101	3·29	2·64	182
Plaice	2·665	0·207	3·19	1·91	182
Haddock	2·962	0·167	3·31	2·52	361
Whiting	2·912	0·185	3·35	2·35	365
Saithe	2·923	0·137	3·32	2·54	241
Ling	3·020	0·118	3·36	2·70	271
Hake	2·871	0·143	3·28	2·52	183

Using Duncan's New Multiple Range Test[9], it can be shown that the results for saithe, whiting and cod are not significantly different from each other, otherwise all differences are significant. Figures 1 to 7 show the nitrogen

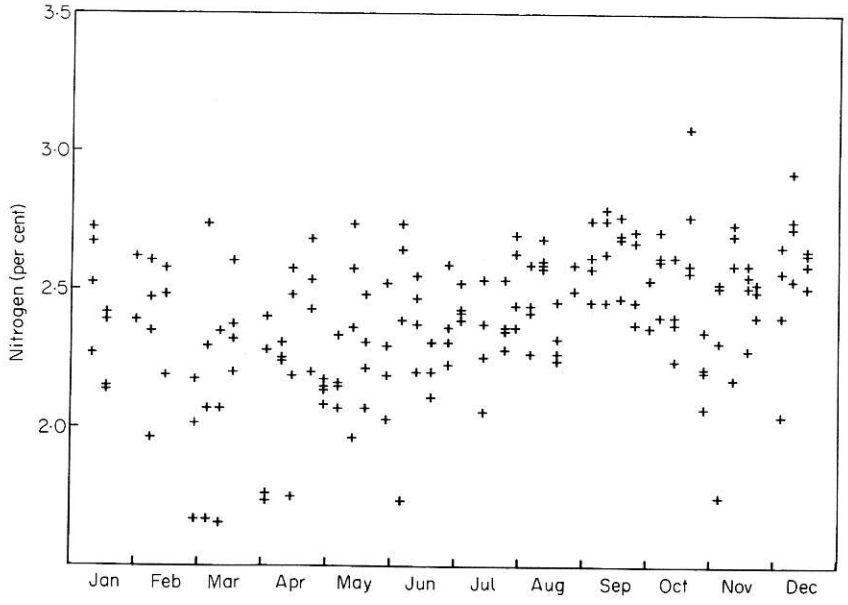

Fig. 1. Nitrogen content of plaice at different times of the year.

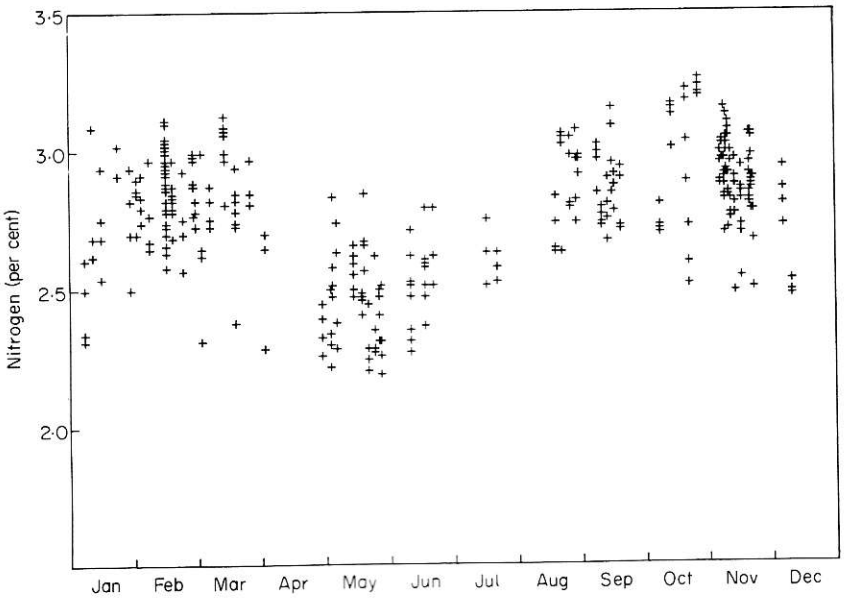

Fig. 2. Nitrogen content of haddock at different times of the year.

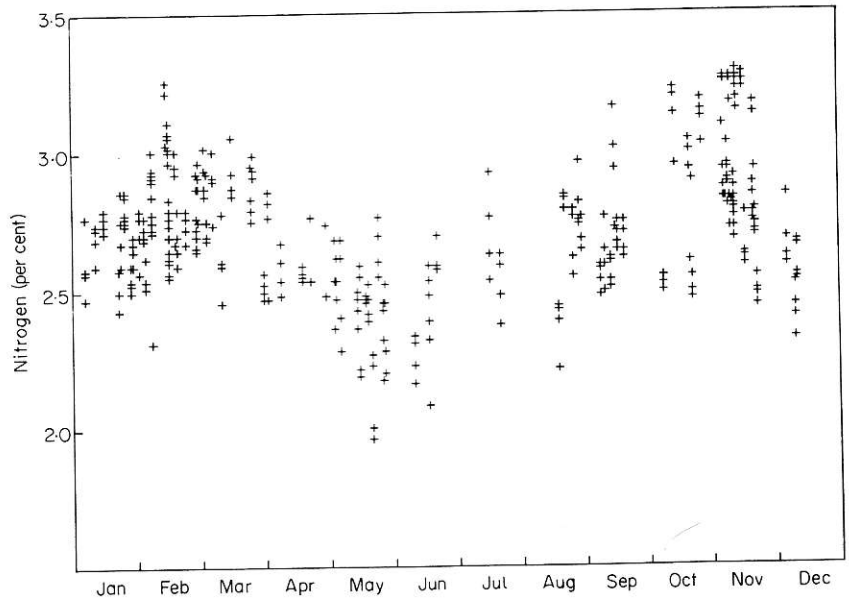

Fig. 3. Nitrogen content of whiting at different times of the year.

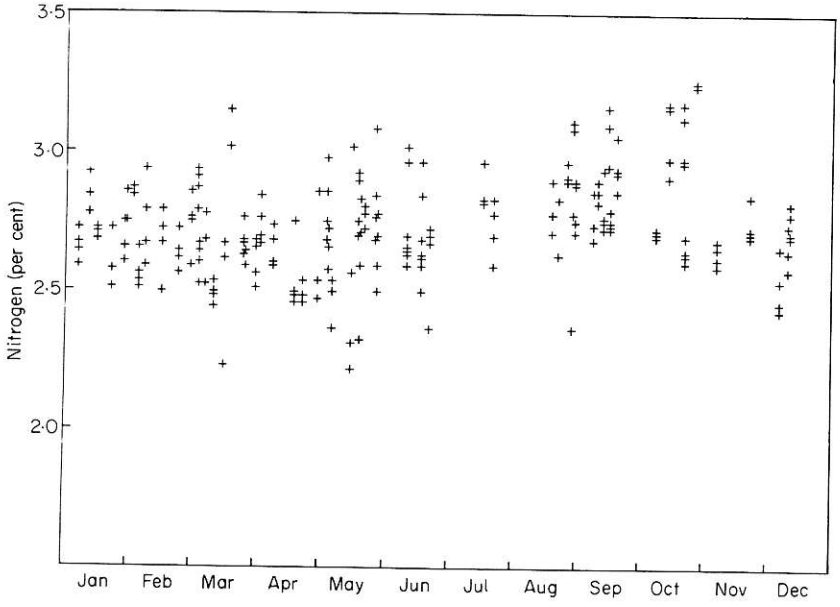

Fig. 4. Nitrogen content of saithe at different times of the year.

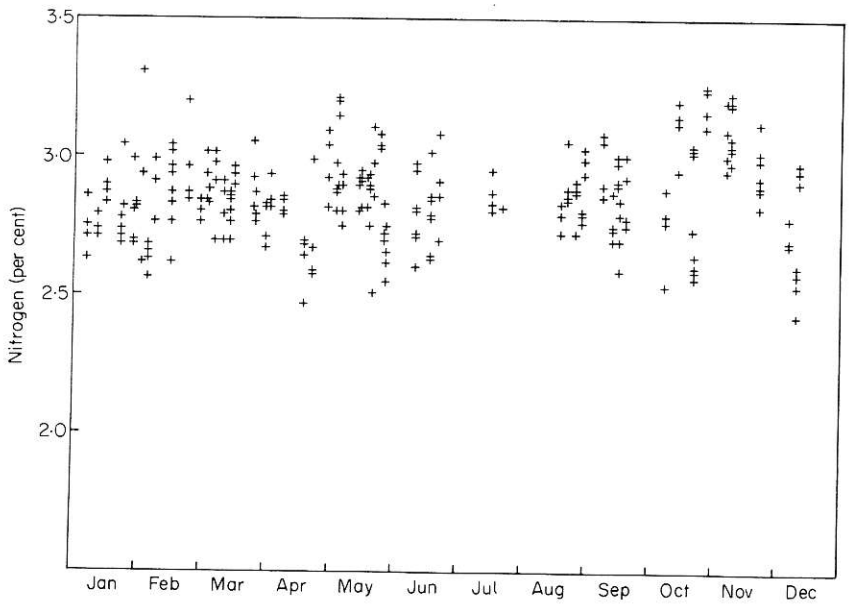

Fig. 5. Nitrogen content of ling at different times of the year.

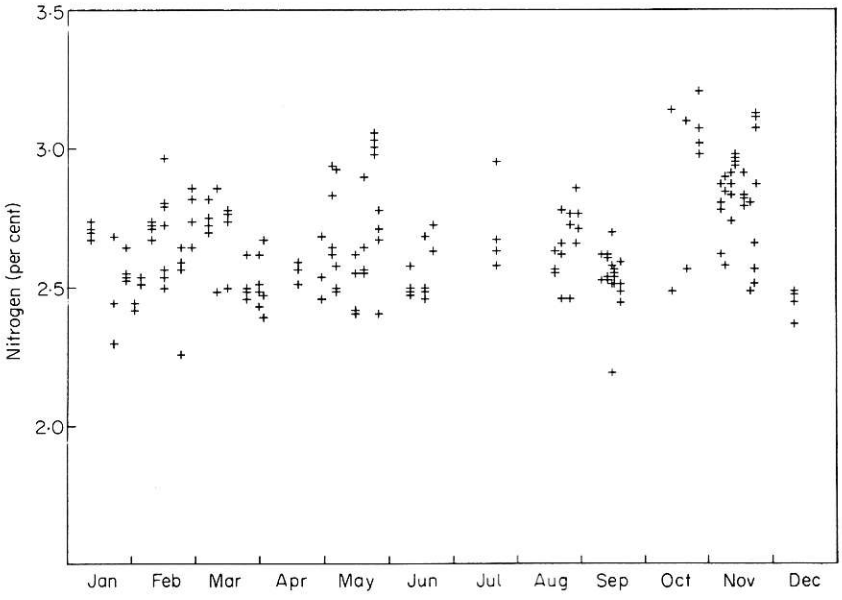

Fig. 6. Nitrogen content of hake at different times of the year.

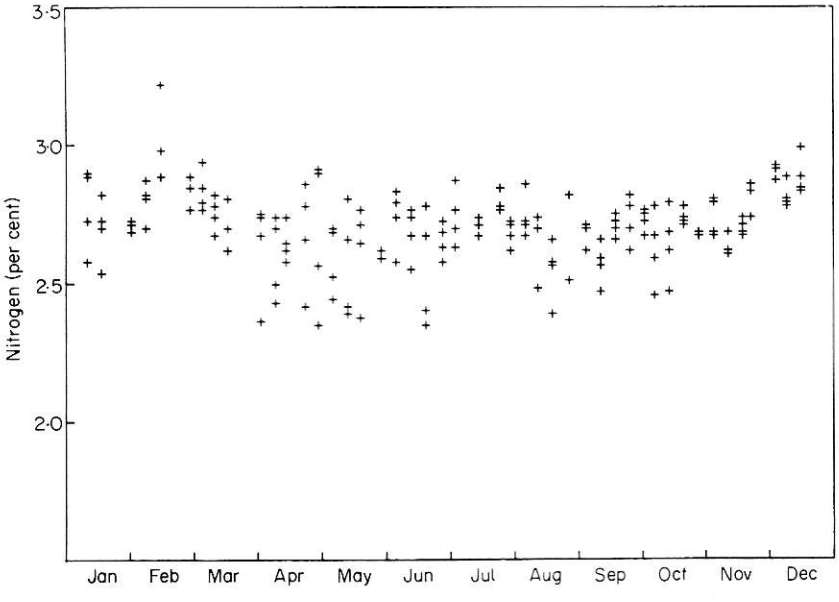

Fig. 7. Nitrogen content of cod at different times of the year.

contents for all seven species plotted against month. It will be noted that the nitrogen content of plaice is more variable than that of the other species. The variation within each batch of four fish caught at the same time in the same place is often surprisingly high.

Haddock and whiting display a very clear seasonal variation. In the month of June, the average nitrogen content of whiting was 2·69 per cent. while in November it was 3·09 per cent. Plaice displays no apparent seasonal effect. In the remaining species it is possible to select, by eye or by statistical test, periods when the average nitrogen content is rather higher or lower than during the rest of the year. For example, cod between April and October tends to be lower than in the remaining months, while saithe, ling and hake appear to have relatively high values for a period within the months September to December.

It is possible that, because fish from different grounds spawn at different periods, the plot of all the nitrogen results against date may conceal more definite seasonal effects in particular stocks. Plotting the results for cod separately for example, for the two fishing grounds most fully represented, does not in fact show up any more distinct seasonal variation.

Correlation and regression analyses were performed to investigate whether nitrogen content in each species was influenced by ground or size. For saithe, ling and hake there was no effect of either ground or size. With cod and plaice there were no differences between grounds. The coefficient of correlation between length and nitrogen content was −0·24 for cod and −0·25 for plaice (in both cases statistically significant at 1 per cent. For haddock and whiting, the correlation within most grounds was not significant, but the mean nitrogen contents showed significant variation between grounds (at the 0·1 per cent. level).

The actual size ranges for each species are given in Table II.

TABLE II

MEASURED LENGTHS OF FISH IN SURVEY OF NITROGEN CONTENTS

	Mean *cm*	Standard deviation	Maximum value *cm*	Minimum value *cm*	Number of fish
Cod	51·2	7·2	69·0	39·0	182
Plaice	39·7	3·2	50·0	32·0	182
Haddock	37·8	6·8	66·0	28·0	361
Whiting	36·8	5·3	52·0	27·0	365
Saithe	46·1	11·4	100·0	32·0	241
Ling	60·2	11·7	95·0	52·0	271
Hake	47·0	9·5	84·0	30·0	183

As was mentioned above, other analyses, not reported here, were carried out. Figure 8 shows the nitrogen content of cod plotted against the water content. It is clear that the correlation between the two values is not sufficiently high to enable the water content to be used to predict nitrogen content or vice versa.

The unpublished data for water, ash and fat can be combined with the nitrogen data to calculate the protein factor that is used in estimating proximate

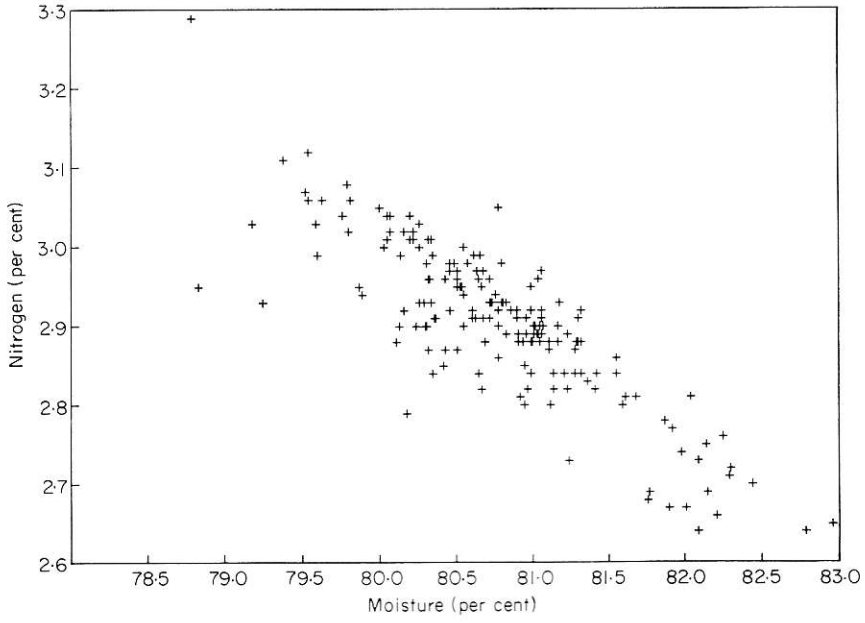

Fig. 8. Variation of nitrogen content of cod with moisture content.

composition. Traditionally a value of 6·25 has been used, although it is known that the factor for fish is probably lower. The present study has produced mean factors shown in Table III.

TABLE III

SURVEY OF NITROGEN CONTENT OF FISH: PROTEIN FACTORS
(Calculated from nitrogen contents and unpublished data for Water, ash, and fat)

	Mean protein factors	Standard deviation
Cod	5·970	0·147
Plaice	6·052	0·238
Haddock	6·007	0·294
Whiting	5·900	0·256
Saithe	6·038	0·302
Ling	6·049	0·305
Hake	6·276	0·306

Finally, the mean nitrogen contents determined in this work can be compared, Table IV, with the mean values and recommended values of the Analytical Methods Committee for cod and saithe[5,6], and with the Committee's collected values[7].

TABLE IV

NITROGEN CONTENT OF FISH: COMPARISON OF NITROGEN CONTENTS FOUND WITH PUBLISHED DATA

Species	This survey N *per cent.*	Analytical Methods Committee N *per cent.*		
		Measured mean	Recommended value	Collected values*
Cod	2·906	2·871	2·85	—
Saithe	2·923	2·937	2·90	—
Plaice	2·665	—	—	2·51–3·02
Haddock	2·962	—	—	2·33–3·25
Whiting	2·912	—	—	3·04–3·18
Ling	3·020	—	—	2·63–3·56
North Atlantic Hake	2·871	—	—	2·64–2·98

* See reference 7.

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A Note on some Concentrated Animal High Protein Foods

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Various forms of concentrated animal protein have been, and still are, in use in various parts of the world, either as food stores for winter use, or as a means of sustenance when travelling, especially in inhospitable conditons. Pemmican is one of the best known foods of this type and was used by the North American Indian. A ball of pemmican, considered to be over 100 years old, may be seen in the Museum at Vancouver B.C., but a sample was not made available for analysis. A recipe for pemmican uses moose, elk or deer meat cut thinly into layers and hung on to a rack to dry in the sun. A fire or rather a smoke smudge is made to keep insects off. After a few days the meat becomes dry, and brittle, and cracks. It is put into a cloth and pounded till powdery, when grease or dripping is worked in to form into balls, dried berries being sometimes added to flavour. Buffalo, bear and caribou are also used. Nowadays many of these preparations may be purchased commercially and are known as "Jerky", with an adjectival description of the meat used.

In South Africa similar preparations to "Jerky" are used and are known as "Biltong", the meats usually being dried with spices. No firm recipe appears to be used, the meat of any available type being cut into strips and dried at a low temperature with selected spices. Ostrich, beef and antelope are the most common.

No similar preparations appear to have been used in Australia or New Zealand, but in Switzerland a smoked, dried ham is cut into almost transparent slices for sustenance.

Samples of various types of "Jerky" and "Biltong" have been purchased in Canada and South Africa from commercial sources and examined by the conventional methods of analysis[1]. The results are shown in the following table. As a matter of interest a calculation of the calorific value of these foods was made and is included. The conventional factors for this calculation, i.e. carbohydrate × 3·75, protein × 4·0 and fat × 9·0, were used[2,3].

For comparison similar analyses of samples of fresh minced beef were made.

From the standpoint of calorific value, bearing in mind the very limited number of samples examined, the Canadian Jerky is superior to the various Biltongs, largely because of it's higher fat content, making it also somewhat more complete. It is also superior to the fresh minced beef, although if compared on an equivalent moisture basis, the positions are reversed. However, when palatability is considered, minced beef is superior to the others, although a spicy Biltong may be attractive to chew.

COMPARATIVE ANALYSES OF CONCENTRATED MEAT FOODS AND FRESH MINCED BEEF

	Beef Jerky			Biltong				Minced beef		
				Ostrich						
	1	2	3	1*	2	Kudu	Beef	1	2	3
Moisture *per cent.*	18·8	17·6	10·1	15·7	10·2	15·0	21·9	64·5	69·2	67·0
Fat *per cent.*	22·7	18·3	39·2	5·1	1·2	1·6	8·0	20·5	10·5	14·3
Ash *per cent.*	8·5	8·8	6·9	5·3	5·1	6·7	8·3	0·7	1·0	0·6
Protein *per cent.* (N × 6·25)	49·8	54·8	37·1	72·5	79·0	74·4	60·6	15·3	18·6	17·8
Carbohydrate *per cent.* (by difference)	0·2	nil	6·7	1·4	4·5	2·3	1·2	nil	nil	nil
Calorific value K cal/100 g	404	384	526	341	344	316	319	246	169	200

* This sample of Ostrich Biltong contained coriander seeds.

References

1. Egan, H., Kirk, R. S., and Sawyer, R., "Pearson's Chemical Analysis of Foods". 8th edn, Churchill Livingstone, London, 1981, pp. 403–404.
2. Paul, A. A., and Southgate, D. A. T., McCance and Widdowson's "The Composition of Foods", 4th edn, HMSO, London, 1978, p. 9.
3. "The Food Labelling Regulations 1984", S.I. 1984, No. 1305.

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Book Reviews

THE VISUAL DISPLAY OF QUANTITATIVE INFORMATION. By Edward R. Tufte. Graphics Press U.K., P.O. Box 8, Godalming, Surrey, 1986. Price £25.00.

I found this book very interesting and the subject was treated extremely well. In particular I was pleased to see sound advice on the reduction of "non-data ink". Professor Tufte deals thoroughly with all aspects of the visual display of information from straightforward graphical excellence through the detection and avoidance of deception to the aesthetics and techniques used in data-graphical design. There were also some good ideas on how best to display information with the minimum of distraction. Although the book does make interesting reading I doubt whether it presents good value for a public analyst laboratory.

J. FULSTOW

CHEMICALS AND SOCIETY: A GUIDE TO THE NEW CHEMICAL AGE. By Hugh D. Crone. Cambridge University Press. 1986. Price Hardback £25.00; Paperback £8.95. 240 pp. plus index.

Incidents such as the disasters at Bhopal and Chernobyl have reinforced the mistrust of anything "chemical" which is felt by substantial numbers of people. The more recent severe chemical pollution of the Rhine will have added to such fears, and the vehemence with which so many adverse opinions are expressed about the addition of chemicals to our food and water, and the pollution of the air we breathe, is indicative of a widespread unease.

So many extreme statements have been made that it is a relief to see a book in which a serious attempt is made to give a balanced view of the risks and benefits of the "Chemical Age" in which we live.

The author, who heads the Personal Protection Group at the Materials Research Laboratories, Melbourne, Australia, addresses himself to "intelligent people who have problems in assessing chemical matters", and seeks to avoid prejudice, self-interest and obscurantism.

After a brief description of the "Chemical Age" and some of its more obvious benefits, we have a basic chapter on "Toxicity and Dosage", in which the difficulties of toxicity testing and of dose–response relationships are discussed, and the statistical nature of the risks brought out. This is followed by a consideration of the routes of entry of chemicals into the body, and of the routes of elimination. The author manages to cover a lot of ground, and suggests sources for further reading.

The next chapter is of special interest in that it deals with analysis. The ability of modern techniques to estimate ever smaller traces of chemicals, and problems arising from this sensitivity, are discussed, together with the statistical significance of results.

A general discussion of chemical hazards, set in the context of the many other hazards already existing, is followed by a consideration of the more specific topics of carcinogenic chemicals, drugs, chemical weapons, and chemical pollution of the environment.

A chapter on protection against hazards, particularly in the workplace, reflects the author's own specialisation. The reviewer is not clear why this should have been placed before two chapters in which more detailed consideration is given to the subject of herbicides. An account is given of problems associated with 2,4,5-T and Agent Orange, and the author presents his views on the public's perception of these problems, with an emphasis on the need for accessible and intelligible information. He also gives in some detail his views on the use of Agent Orange. The closing chapters deal with regulatory aspects, natural hazards, chemicals in food (including food faddism), and sources of information about chemicals.

While it is obviously impossible for a book of this size to cover all these topics in depth, each of the chapters contains references for those wishing to dig deeper. In the reviewer's opinion, the author has made a valiant attempt to give a broad and balanced view of a very extensive field, and his book deserves to be widely read, by the professional scientist as well as the interested public.

M. C. FINNIEAR

CONTRIBUTION OF CLUSTERS PHYSICS TO MATERIALS SCIENCE AND TECHNOLOGY. Edited J. Davenas and P. M. Rabette. NATO/ASI Series E. Mortimus Nyhoff Publishers, Dordrecht, 1986, £69.25. 646 pp.

This rather weighty tome may be of interest to some analysts but they are likely to be few. The title may be somewhat deceptive for many analysts, although the chapter by Moisar from Agfa-Gevaert dealing with the properties of clusters in the photographic process may be of use in some laboratories.

A page rather interestingly is devoted to "Physics in the Beach", or the "Theory of Windsurfing", but as the next page is blank, it is assumed this has been included as a stop gap, i.e. to fill up space. It is somewhat reminiscent of second form physics, being a resumé of the triangle of forces and the parallelogram of forces.

The value of the copy sent for review is reduced by the absence of pages in some chapters, e.g. pages 414, 415, 418, and 419 are missing from "Optical Properties of Small Particles".

There is a very full and useful chapter on the "Theory of Percolation".

No doubt the book (when complete) may be of value to workers in material science, but I cannot frankly recommend it for the library of a Public Analyst's Laboratory.

G. V. JAMES

ATOMIC ABSORPTION SPECTROMETRY. By Bernhard Welz. Second Edition. English Translation by Christopher Skegg. VCH, Weinheim, 1985. Price DM 160 (£49.00). 506 pp.

Atomic Absorption Spectrometry (AAS) is a technique which is surely in daily use in the laboratories of all Public Analysts. The appearance, therefore, of a new edition of a book which sets out to serve as both a text book of the fundamental principles and a source of reference for methodology and applications should be of interest to all members of the profession.

The present volume is the second English edition, being a translation of the third German edition, published in 1983.

An introductory chapter is followed by one on radiation sources, and then by a substantial chapter on atomisers, which gives an historical survey of, and an introduction to, the various techniques (flame, graphite furnace, hydride generation, and cold vapour), including some consideration of solid sample introduction. Much of the discussion in this chapter is considerably extended in a later chapter, entitled "The Techniques of Atomic Absorption Analysis", which is new to this edition, and is a means adopted by the author of adding new material. The new chapter gives a good account of the development of these techniques up to the beginning of the eighties. However, it seems to the reviewer that the splitting of the material beween those physically separated chapters does somewhat distort the pattern of the book.

Other chapters include one on "Nomenclature and Techniques". The nomenclature accords with the recommendations of IUPAC and ISO. The use of the word "techniques" in the heading could confuse, but does in fact cover calibration techniques, and separation and enrichment techniques.

Spectral and non-spectral interferences are covered, with methods for correction and/or elimination.

The "text book" section closes with a brief consideration of the related methods of atomic-emission spectrometry (both flame and graphite-furnace), and atomic fluorescence spectrometry.

The remainder of the book, consisting of only two chapters, nevertheless constitutes about one-third of the book.

The first deals individually with the elements that can be determined by AAS, advice being offered on the pros and cons of the various methods. The final long chapter deals with specific applications in various fields, including, *inter alia*, food and drink; agricultural analysis; the environment; and clinical analysis. These two chapters bring together a considerable amount of information in readily accessible form.

The book is well-referenced, the references being consolidated at the end. There are, in fact, two blocks of references, each listed alphabetically, the first series ending at 1388, and the second beginning at 2000. This is obviously the device used for referencing the new material, and perhaps a single list will appear in a future edition. The references go through to 1981, with at least one for 1982, which seems reasonable, bearing in mind the date of the German edition (1983).

Though fairly closely printed, the book is easily readable, and its 506

well-bound pages form a substantial volume which should stand up well to regular use. It should prove a useful addition to the libraries of those analysts who are seeking an up-to-date treatment of the subject.

M. C. FINNIEAR