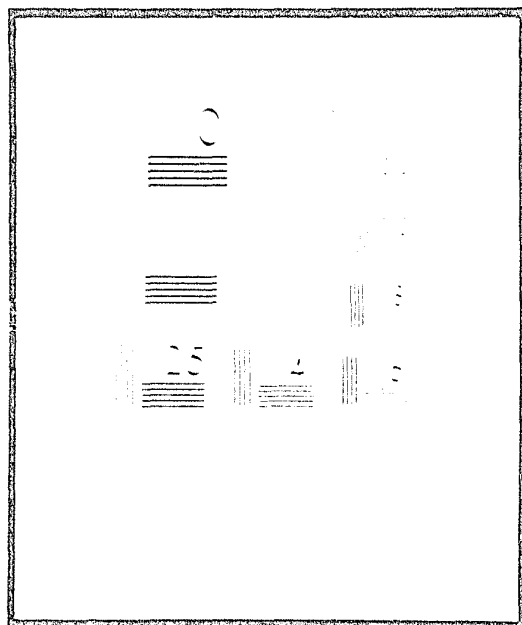


NCJRS

The NCJRS was created from documents received to
provide the NCJRS with data. Since NCJRS cannot afford to
store all the data it receives, it has developed a system
to store the data in a way that will allow the user to
retrieve the data that is needed for his or her work.



When using the NCJRS, users should be aware of the following:
1. The NCJRS is not a search engine.
2. The NCJRS is not a database.
3. The NCJRS is not a file server.

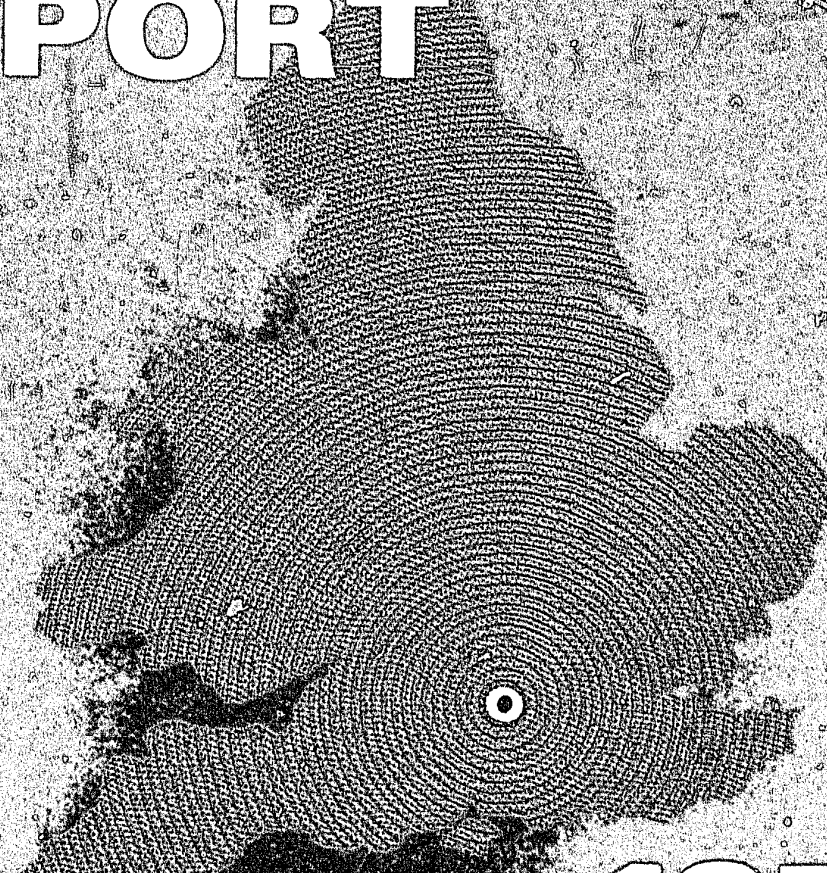
For more information on the NCJRS, please contact the
National Criminal Justice Reference Service, 440
U.S. Department of Justice, Washington, D.C. 20531.

U.S. DEPARTMENT OF JUSTICE
LAW ENFORCEMENT ASSISTANCE ADMINISTRATION
NATIONAL CRIMINAL JUSTICE REFERENCE SERVICE
WASHINGTON, D.C. 20531



HOME OFFICE CENTRAL RESEARCH ESTABLISHMENT

ANNUAL REPORT



1975

32688

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1. INTRODUCTION

The Establishment has increased in size both physically and in the number of staff, since the writing of last year's Annual Report. Information and Contracts Divisions moved into a new building at the beginning of the year and the scientific staff rose in number from 40 to over 50: all Divisions are now almost up to strength.

Research in serology has taken great strides this year with the successful introduction of automated machines capable of quantitatively grouping blood, saliva and semen in the ABO system at a rate of about one sample a minute. This has had practical application in operational work and has also opened the door to studying the secretor status of individuals, the variation between fluids and within fluids and the stability of antigens in stains. The work on immunological techniques to discriminate between blood from different individuals on the basis of their age, disease history and allergen status marks the successful beginning of work designed to tell the police officer more about the offender than his blood group.

In the field of poisons and drugs, several poisons, that were previously considered to be virtually undetectable, have been successfully revealed and the demonstration of LSD and cannabis in blood is now approaching the operational phase. The complexity of these analyses prevent them being used as a routine method in police investigations so far, but the fact that the drugs are present in amounts of about 1 part in 1000,000,000 will indicate to the lay reader that this is a very difficult technical area.

The appointment of a new Head of Chemistry Division, after a period of major staff changes, has brought new impetus. The installation of a new CI/EI mass spectrometer, a computerised data bank for mass spectra, solid work in atomic absorption techniques following spark source mass spectra studies, as well as fundamental work on soil analyses and on explosive residues on hands has resulted in the Division being in the forefront of forensic chemical analytical techniques.

Information Division continues to provide an essential service for Regional Laboratories with about 100 queries a month being answered directly relating to operational case work; a detailed appraisal of the use of Facsimile, Telex and direct computer links is well under way. The building up of data banks on headlamp and spot-lamp glasses as well as footprint and tyre patterns has continued together with a collection of analytical data on drugs and poisons.

This year saw the first "Consultative Document on Research" being collated by the Forensic Science Branch of the Home Office. This book brought together all research being done in Regional Laboratories, the Metropolitan Police Laboratory and by CRE both "in house" and by external contracts. It also provided the forum for discussion with police forces of the work proposed for 76/77. Full note has been taken of the most helpful suggestions made by the Police in drawing up a definitive research programme.

It is nearly ten years since the Establishment was founded and this year has seen the departure of three members of staff who joined in the earliest days. Mr S Brandish, who was responsible for all the Xerox copying in Information Division, retired, Mrs I L White, the Director's Personal Secretary was promoted and left to join the Staff of the Police College, and Mr S Jones, Clerical Officer, who steered the Establishment's Administrative Work through its early days retired on medical grounds. We are most grateful to them for their dedicated service and wish them well.

The end of last year saw the departure of Mr V Emerson for 2 years on a Senior Professional Administrative Training Scheme (SPATS) and Dr A Scaplehorn returned in October from his SPATS course.

The introduction of administrative training for scientists has been a feature of the last few years and, although the Scheme is still at an early stage, the signs are encouraging.

This is the last Annual Report of CRE for which the current Director is responsible, as early next year he takes up the appointment of Controller of the Forensic Science Service of the Home Office. Mr S S Kind will replace Dr A S Curry as Director of CRE.

CRE depends on the support given to it by many other organisations - too numerous to mention individually but to all of them we express our thanks. Special mention must be made to the staff and Directors of the Regional Forensic Science Laboratories and the Metropolitan Police Laboratory and the Inter-Laboratory Advisory Committees. The help of the Director and staff of the Atomic Weapons Research Establishment is also gratefully acknowledged.

2. STAFF DETAILS

We Welcome:

Mr A R Allan (HSO) from University of Newcastle-Upon-Tyne
Mr E M Besly (SO) from the British Museum
Miss C M Bosley (ASO) from Westwood School, Tilehurst
Mr R Burgess (ASO) from Theale Green School
Mrs M Chapman (Cleaner) from Suffolk Police Authority
Suffolk
Mrs M Dorrill (SO) from Brunel University
Miss P Hale (Trainee Typist) from Willink School, Burghfield
Mr M D Hammond (SO) from the University of Dundee
Miss J Home (ASO) from Kendrick School, Reading
Mr I J Humphreys (HSO) from Home Office FSL Aldermaston
Miss A Livingstone (Typist) from Willink School, Burghfield
Dr R Macrae (HSO) from the Institute of Molecular Biology,
Zürich
Miss A Mair (Typist) from Willink School, Burghfield
Mr B Platt (ASO) - from AWRE, Aldermaston
Dr A Scaplehorn (PSO) on return from F3 Division Police
Department, Home Office
Dr A T Sullivan (SRF) from Cardiothoracic Institute, London
Mrs P Tew (Typist) from Nuclear Enterprises Limited, Beenham
Dr J Twibell (HSO) from Charing Cross Medical School, London
Mr P L Williams (HSO) from the Forensic Science Laboratory
Bristol

Departures

Dr Janet Drayton (SSO) left to join the staff of the
Metropolitan Police Laboratory, London
Mrs A Golding (Typist) left to join the staff of the
Home Office Forensic Science Laboratory, Aldermaston
on acting promotion to Personal Secretary

Mr C Handoll (PSO) left to join the staff of the Home Office
Forensic Science Laboratory, Chorley

Mrs S Webb (Audio/Typist) left to have a baby

Mrs I White (PS) was promoted to EO and went to join
the staff at the Police College, Bramshill

Congratulations to:

Dr A T Sullivan who married Dr Sandra Vellucci

We record the attachment of the following vacation
student: Miss C Rushton, from Southampton University;
also the following Sandwich Course Students:

Miss M Burkett from Hatfield Polytechnic; Miss A Ward from
Trent Polytechnic, Nottingham; Mr G G F Cadwallader from
Loughborough University and Miss A E P Gorvin from Surrey
University.

Retirements

Mr S Brandish (CA) who had been in CRE since its inception
In December 1966 retired at the end of September

Mr S Jones (CO) retired on medical grounds at the end of
December

Promotions:

Mrs B Beattie to SO

Dr R Dudley to SSO

Miss T Holdstock to SO

Dr A E Kipps to SSO

Mrs P Tew to acting PS

Appointments and Qualifications

Mr C Howden obtained Graduateship of the Royal Institute of Chemistry (GRIC).

Mr D Osselton has obtained a PhD from London University

Miss V Quarmby gained her BSc from London University

Mr M Swain was appointed Chairman of the Chemical Society Chemical Information Group

Dr D J Werrett gained his PhD from Birmingham University

Registered for Higher Degrees

Mr P Owen has been accepted for PhD at Guildford (University of Surrey)

Mr J Sutton for PhD at Reading University

Mr P L Williams - M Phil, (University of Surrey)

3. LECTURES GIVEN AND CONFERENCES ATTENDED BY STAFF;
OVERSEAS VISITORS

Lectures given by Director and Staff

In February Dr Curry visited Canberra, Australia at the invitation of the Attorney General's Department to act as Consultant. In the course of this visit he lectured at the 4th National Symposium on Forensic Science in Perth and the Victoria & New South Wales Branches of the Australian Forensic Science Society. He visited the Australian Department of Health and numerous organisations concerned with forensic science and pathology as well as Police Forces and Customs Authorities in Melbourne, Sydney and Adelaide. In addition visits were paid to the Department of Pathology and the Department of Chemistry in Singapore. Dr Curry wishes to acknowledge the hospitality and courtesy extended to him on this Journey.

Members of staff have given lectures to the following bodies: Racecourse Security Services Laboratory; Bramshill Police College; Scottish Police College; Royal Military College of Science; Metropolitan Police Laboratory; North Staffs Medical Institute; Chemical Society Meeting at Aldermaston; Pharmaceutical Society, Reading; West Yorkshire Metropolitan Police Academy; National Conference of Drugs Squad Officers Seminar at CDIIIU London; Chemical Society Meeting in London; German Society of Forensic Serology, Innsbrück; University of Kent Biological Society; Guy's Hospital; 4th Annual Meeting of the Committee of Forensic Tyre Examiners, and Tyre and Allied Industries Manufacturers.

Conferences Attended by Staff

Dr A T Sullivan, Dr P Twitchett, Mr P Williams, Dr S Fletcher, Dr A C Moffat, Mr P Owen and Mr D Osselton attended a Conference on "Forensic Analysis" organised by Chemical Society; Dr P Twitchett, Dr A C Moffat and Mr P Owen attended a Conference on the "Moorgate Disaster" at Wood Street Police Station by the Forensic Science Society; Dr P Twitchett attended a Conference on "Use of HPLC in Clinical Chemistry" in Watford; Dr S Fletcher attended a Conference on "Radioimmunoassay" Oxford and an International Congress on "Liquid Scintillation Counting" in Bath; Dr Moffat attended an International Conference on Traffic Medicine in London and a course on "Laboratory Safety" in London; Miss V Quarmby attended a Course on

"Fibrous Protein Structure" and a Symposium on "Comparative Biology of Skin" at the Zoological Society of London; Dr D Werrett attended a Discussion Group on "Anaphylactic Antibiotics and Antigens at the Biochemistry Society and British Society Immunology and a Conference with Dr A E Kipps on "Immunology" and a Conference on "Immunological Aspects of Haematology" organised by the IMLT at Harrogate with Dr A E Kipps; Dr A E Kipps attended a Symposium on "Sexual Offences" at Leeds; Dr P H Whitehead attended a Symposium on "Sexual Offences" organised by the Forensic Science Society in Leeds. Mr P Owen and Dr D Osselton attended a Course on "Assay of Drugs and Other Trace Substances in Biological Fluids" at the University of Surrey; Mr J Porter attended a "Colour Symposium" at Salford College of Technology; Mr C Brown attended an Instem Laboratory Users Group at the University of York; Dr A Scaplehorn attended a Conference on "Microfilm and the Computer" in London; Dr R Ardrey attended the 8th Meeting of British Mass Spectrometry Group at the University of Keele; Dr J Twibell attended a Chemistry Society Analytical Division Conference on "Data Acquisition and Processing" at Birmingham University; Dr J Drayton attended the Meeting of Biomedical Mass Spectrometry Group at the School of Pharmacy; Mr K Smalldon attended a SAC/AD Meeting on "Simultaneous Multi-element Trace Analysis"; Mr P J Gomm attended a Conference on "Developments in Pharmaceutical Analysis" at the Chemical Society (Analytical Division);

Overseas Visitors

We had 39 visitors from Overseas during the year from 15 different countries.

4. BIOLOGY DIVISION

The work of the Division has continued along the main lines of enquiry as detailed in last year's Report with continued emphasis being given to investigating the problems associated with the grouping of body secretions especially semen.

A new capacity to study quantitatively and automatically the levels of the blood group substances A, B and H on a 3-channel Auto-Analyser, is enabling fundamental studies on the nature and stability of these substances in saliva and semen to begin. The use of the immunoglobulin markers Km(1)[Inv(1)] and Gm(1) in grouping semen and saliva, which has not previously been reported in the literature, opens up a much needed new avenue of discrimination of these fluids and satisfactory typing of semen stains using these markers has been achieved.

The new concept of bloodstain characterisation known as 'antibody profiling' has been extended to include allergens and this approach opens up new possibilities of discrimination. Apart from complementing the discrimination achieved by traditional grouping methods, 'antibody profiling' offers the prospect of gaining quite new information concerning the 'donor' of a bloodstain relating to age, race, clinical history and perhaps style of living eg whether the person has travelled abroad extensively.

Whenever possible the work has been directed to simplifying present techniques used in forensic biology under the general heading of "Work simplification" and, in addition, a new programme of research work on hair has been initiated.

For convenience the work is described below under four main headings:

A Fundamental Studies

(i) Semen Enzymes

Last year's report described stability studies on PGM in liquid semen and semen stains which noted a progressive loss of activity. In addition 'anomalous' patterns unrelated to any known PGM phenotype have been confirmed on further studies of liquid semen from vasectomised men.

All of these experiments were based on visual examination of starch-gel electrophoretic plates. Efforts this year have been directed towards quantifying the PGM assay in semen using a reaction-rate analyser in order

to make a more precise study of the stability of PGM in both semen and semen/vaginal secretion mixtures. Early investigations in this area suggest that the present concentration of NADP as utilised in the conventional agar overlay used for developing PGM on starch-gel plates is inadequate to compensate for the high level of NADP-ases found in semen (CRE Report No 173).

(ii) Saliva Enzymes

The development of the amylase sensitive paper previously described (CRE Report No 120) allows the screening of large areas of garments for saliva. A large number of apparently unstained 'clean' clothes from members of staff have been screened and many amylase positive areas have been identified on these clothes especially round the collar and lapels, cuffs and pocket flaps (Fig 1) CRE Report No 156. Investigations are underway to confirm the nature of these 'spots' presumed at present to be saliva droplets and, of equal importance, to assess their significance in the grouping of bloodstains in the ABO system. It is possible that this "natural" background of saliva droplets on a garment could give rise to difficulties in the interpretation of grouping results from bloodstains on clothes.

Further fundamental investigations on iso-enzymes of amylase in saliva are now the subject of an External Contract.

(iii) Immunoglobulin Markers in Saliva and Semen

The immunoglobulin variants, Gm and Km(Inv) have been used for some years for the grouping of blood and bloodstains in forensic biology. The very good discriminating power of these systems, and the simplicity of the inhibition techniques used, make Gm and Km grouping an attractive proposition. In view of the limited capacity of the biologist to discriminate saliva and/or semen relative to blood, a new grouping system, applied to semen or saliva would be valuable. In view of this attention was turned to the detection of immunoglobulin markers in body secretions.

Immunoglobulins are found in other body fluids besides serum although generally at much lower levels. However, a consideration of the very small quantity of blood required to perform these tests led to the belief that it might be possible to Gm and Km group semen and saliva.

MAN'S JACKET

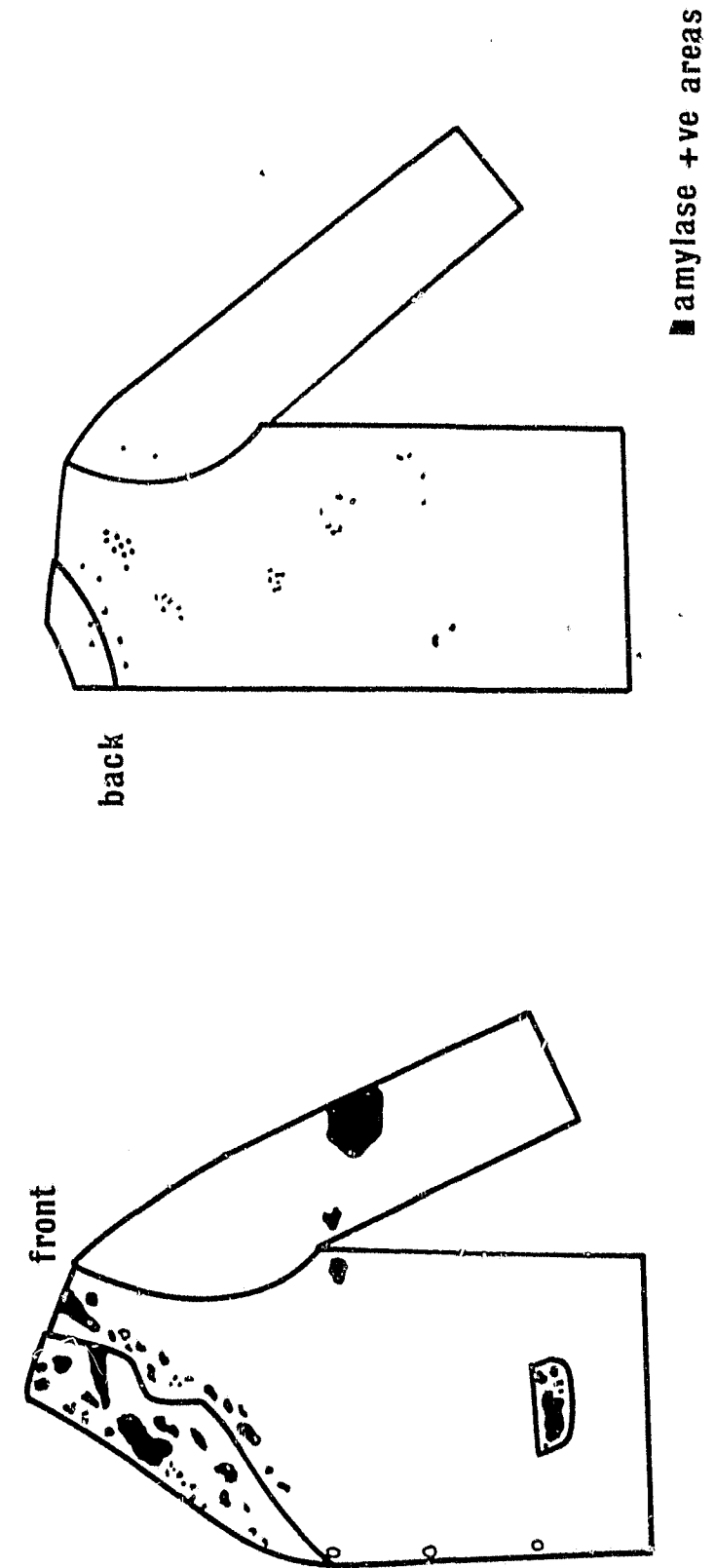


Fig 1. Saliva Stains, located by testing an apparently unstained garment with the amylase-sensitive test paper.

It has been found possible to group both liquid saliva and semen in the Km (1) and Gm (1) systems using a simple inhibition technique after reduction of viscosity of these fluids by the simple expedient of freezing, thawing and centrifuging (CRE Report No 155). Successful grouping of semen stains has also been achieved. Investigations are under way into the use of other Gm markers.

The potential discriminating power of the Km and Gm systems combined is considerable as can be seen from the following table:

Phenotype				Frequency (BRITISH)
Km(1) [Inv(1)]	Gm(1)	Gm(2)	Gm(5)	
-	-	-	+	34%
-	+	-	+	25%
-	+	+	+	14%
+	-	-	+	7%
+	+	-	+	5%
-	+	+	-	5%
-	+	-	-	4%
+	+	+	+	3%
+	+	+	-	1%
+	+	-	-	1%
-	-	-	-	0

If the reliability of the system can be proved, and the long term aim is to test this, then the capacity of the biologist to discriminate semen stains in sexual offences will be dramatically improved.

It should be noted that the system has an "internal control" in that Gm (1-5-) individuals are exceedingly rare so that a +ve result for either one or both of these markers must be obtained from stains which are satisfactory for grouping. In effect this eliminates the comparable problem encountered with "non-secretor" grouping in the ABO system.

(iv) Automated studies on ABH substances in secretions

At the present time two 3-channel auto-analysers are in operation each capable of testing up to 60 samples per hour for the three antigens A,B and H [O] simultaneously. Both instruments work on an inhibition principle in a continuous flow system with subsequent lysis of non-agglutinated cells and the resultant free haemoglobin being measured colorimetrically and recorded. As in any inhibition system the concentration of blood-group substances present in a test sample is inversely related to the number of agglutinated indicator cells. Figure 2 illustrates the results from one of these instruments. The "base line" represent 0% inhibition ie minimum colour development from lysis of minimum free cells reflecting maximum agglutination.

One of the first requirements of any quantitative system is the availability of suitable standards. In this instance a freeze dried standard from pooled saliva has been prepared. The standard curve obtained on one instrument for each channel is given in Figure 2. The reproducibility of the instrument using this standard is $\pm 7\%$. The second requirement is to understand the stability characteristics of the items under study. Clearly correlation studies on the absolute level of B substance, for example, in a man's semen and saliva are dependent on the acquisition of suitable stable samples for analysis. In recognition of this the use of one analyser is directed towards making fundamental observations on the stability of AB & H substances in secretions.

Figure 2 illustrates a basic problem already encountered. The results U (Upper) and L (Lower) show the distribution of blood-group substances in the upper and lower layers of a saliva sample after spinning at the relatively low speed of 20,000 r.p.m. in a centrifuge

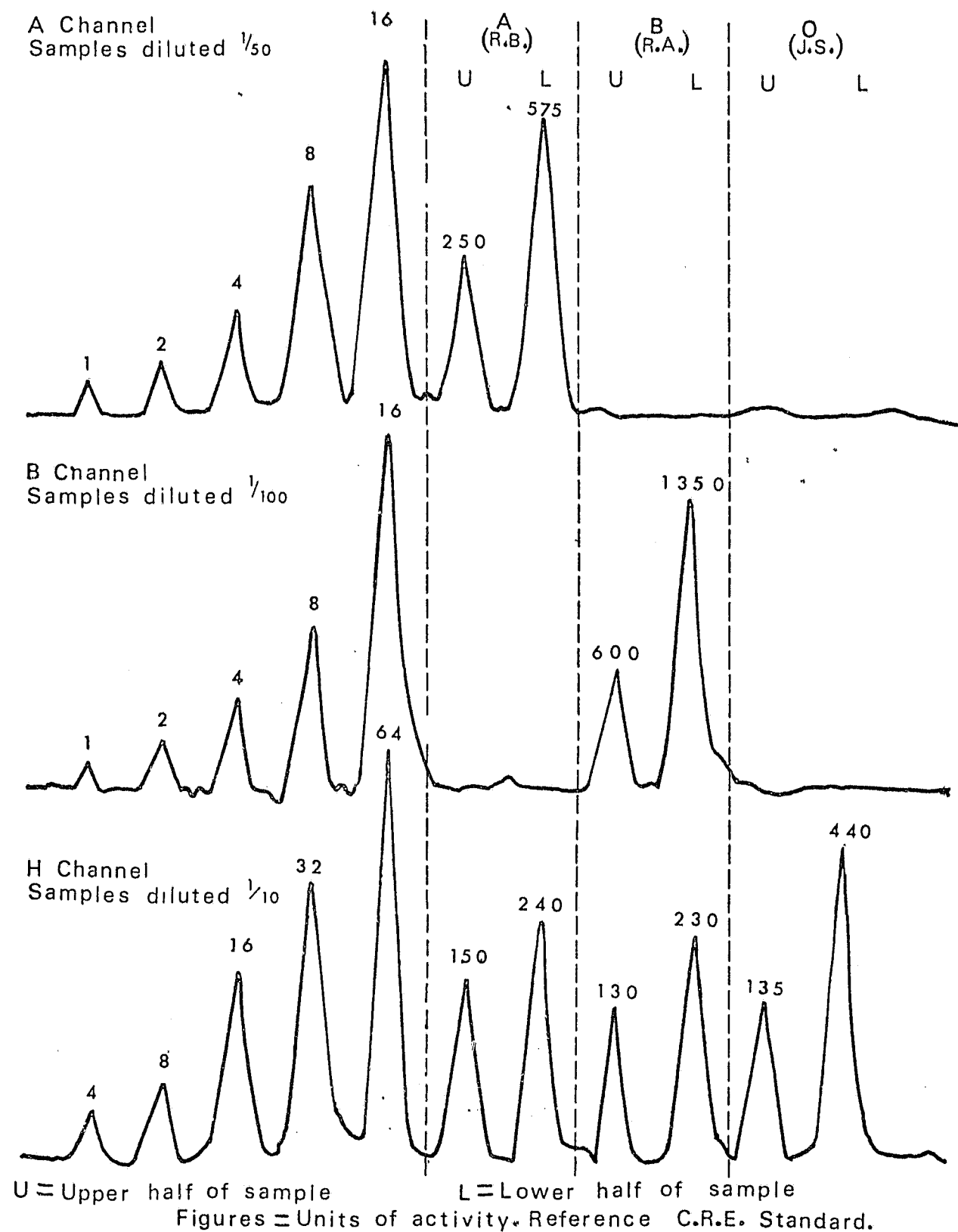


Fig 2. Typical Traces for 3-channel Technicon Auto-analyser showing, with standards, the effect of centrifugation on salivary blood group substances, the saliva having been diluted as indicated.

tube for 1 hour. The heterogeneous nature of saliva and semen must be recognized in any interpretation of "quantitative" results of the assay of blood-group substances in these fluids. (cf amylase in lip-mucus secretion CRE Report No 127).

Studies on the potential genetic polymorphism of A, B and H substances in saliva as reported by Fiori and his colleagues have been carried out utilising the second auto-analyser associated with a gel-filtration column. At present Fiori's observations have not been confirmed.

B Antibody Profiling

An inter-laboratory blind trial, involving the Home Office Forensic Science Laboratories designed to test the capability of differentiating an adult's bloodstain from that of a child was successfully carried out. The technique involves a fluorescent antibody method and allows the detection of antibodies to the bacteria Mycobacterium tuberculosis and Vibrio cholerae. These antibodies only occur in measurable quantities in blood from post-adolescents and in cord blood. It is thus possible to identify a bloodstain as originating from an adult (CRE Report 154).

Microbial antibodies are also found in vaginal secretions. Menstrual blood can often be discriminated from capillary blood of the same donor by showing that the antibody level in the former case is very much higher when related to haemoglobin content (CRE Report No 161).

During the year, the concept of antibody profiling has been extended to include the IgE class of reaginic antibodies associated with many common allergies (eg hay fever). A commercially available immunoradiometric assay (Phadebas RAST) has been modified to measure specific IgE antibodies in bloodstains. The allergy status of an individual can now be diagnosed from a small sample of blood. Such information could be useful to a police investigating officer. In addition such antibodies extend the potential for straightforward discrimination of bloodstains. (CRE Report No 162).

An important feature of antibody profiling is that a very high discrimination may be achieved using just one experimental procedure i.e. an indirect labelled-antibody method.

It is anticipated that the antibody profiling of bloodstains will be further extended into the detection of anti-viral antibodies by means of an External Contract. Once the range of detectable antibodies in bloodstains extends from parasites, through bacteria, viruses and allergens then an individual's clinical history, which will reflect his life-style, may be obtained from a bloodstain.

C Work Simplification

A variety of projects have been investigated under this heading. The separation of seminal acid phosphatase from vaginal acid phosphatase by means of iso-electric focussing in 3½ hours is now being further evaluated in collaboration with Regional Laboratories. (CRE Report No 159). The PGM capsules described last year which provide an 'instant' developing mixture following electrophoresis have been evaluated by various laboratories and found to be useful. In addition the regional forensic science laboratories have been supplied with amylase-sensitive test paper for saliva screening.

Commercially prepared 3,5,3',5', Tetra-methyl benzidine (TMB) has been evaluated as an alternative to carcinogenic reagents for presumptive blood testing and the staining of haptoglobins following electrophoresis on polyacrylamide gel. Although TMB proved an acceptable alternative its purchase price is relatively high compared with the traditional reagents. Other, cheaper reagents are under investigation and leucomalachite green has been found to be an acceptable alternative. (CRE Report No 180).

The anti-human latex for species identification is now commercially available and a few batches of other anti 'animal' latex have been either purchased in bulk or produced at HOCRE for distribution to Laboratories. It is hoped to put the production of these reagents on a more regular basis in future.

D Hair

A new programme of research work on hair is being initiated with the emphasis on a study of the electrophoretic properties of keratin. In addition classical microscopic studies of hair have also been made with respect to the significance of the presence of sheath cells associated with roots. It

has been shown that the traditional view of differentiating a plucked human hair from one naturally shed, based on the presence of sheath cells, requires qualification. Recent work indicates that the presence of sheath cells, associated with a hair root, may be more a reflection of the individual than of the mode of removal (CRE Report No 171).

REFERENCES

1. Fiori et al., J. Chromatog., 1971 55 337-349
2. Idem, ibid 351-363
3. Idem, ibid 365-375

5. CHEMISTRY DIVISION

A MASS SPECTROMETRY

(i) Organic

The Micromass 12B low resolution mass spectrometer has been upgraded during the year to a Micromass 16F. As well as offering improved resolution and sensitivity the new instrument allows both chemical and electron impact ionisation.

The collections of mass spectral data have grown rapidly during the year. A set of normalised spectra for the more commonly encountered drugs has been produced for the regional laboratories using the Carrick interface to the Hewlett Packard 2100 computer (CRE Report 159). A computer-based retrieval system has been built up in which the 8 most intense ions are recorded for each compound. This simple but effective system now contains about 1,000 entries and is being continuously updated. At regular intervals a listing is automatically produced and sent to the regional laboratories for manual searching. The overall aim has been to provide enough data to solve about 90% of their identification problems both quickly and directly. In the 10% of problems which are not so easily solved it is anticipated that CRE will be contacted for assistance.

Three lines of attack are used at CRE to solve the more difficult identification problems. The first stage involves a computer based search of the 8 peak index. The computer rapidly provides sophisticated searches which cannot be performed manually. The second stage involves the use of the commercial Mass Spectral Search System which now contains 31,000 compounds and is available on a remote computer via the Cyphernetics Corporation. The third stage is specifically designed to deal with new compounds or metabolites which are not as yet available as pure samples. The name, molecular weight and structure of such materials are recorded with structure being listed in a form known as Wiswesser Line Notation. The computer file now contains about 2,500 compounds and can be searched using sub-structural information derived from any spectroscopic technique, molecular weight information or chemical name (CRE Report Nos 157 and 169). This system has already led to the identification of several unknowns which had defeated other routes of enquiry.

A mass spectrometry seminar was held at CRE in June which staff from all the regional laboratories attended. Two important points emerged from the presentation of the retrieval systems. The overall aim of identifying 90% of the spectra immediately was being met under operational conditions and there was also a desire to conduct an inter-laboratory experiment in which CI and EI spectra were recorded for 10 unknown compounds. This experiment has now taken place and all the samples were identified without difficulty (CRE Report No 170).

A service facility continues to be offered to both regional laboratories and other divisions of CRE.

During the year 32 cases have been completed for regional laboratories. The caseload has decreased because the Birmingham, Chorley and Metropolitan Police Laboratories' mass spectrometers became operational earlier this year. In view of the regularity with which benzodiazepines, their benzophenones and metabolites are encountered in casework, a collection of their mass spectra and structures has been prepared (CRE Report 174).

A quantitative method for the determination of fluoroacetamide and fluoroacetic acid in tissues has been reported in conjunction with Toxicology Division (CRE Report No 172). Co-operation continues with Toxicology projects, concerned with the action of solvents on drugs and the assay of catecholamines in tissue, and with Drugs of Abuse Division, in connection with cannabinols in body fluids.

(ii) Inorganic

The feasibility study stage has been completed in the project concerned with trace elements in liver tissue. The low temperature asher has been shown to yield good recoveries for all elements of interest with the exception of the halogens, selenium and tellurium. Interfering molecular species are now well understood and more than 40 elements are capable of measurement, 26 of which are of immediate interest to the forensic toxicologist. (CRE Report No 177). Unfortunately mercury appears to be lost during sparking of the electrodes. Alternative and complementary analyses have been developed using flame atomic absorption spectrophotometry and separated liver tissue has been shown to be analytically homogeneous.

A quantitative method is now being developed using the zinc present in the liver, as internal standard. Photoplates have been selected for ion detection and programmes have been written so that the plates can be read automatically using the Computerised Autodensidater. Concentrations of specified elements are finally printed out as shown in the specimen output (Fig 3).

LIVER ASH PROGRAM 30/10/75
 SAMPLE S.151 PLATE 1263 ASH WEIGHT 1.58%
 EXPOSURES TO BE MEASURED:
 30, 20, 10, 7, 5, 3, 2, 1, .6, .3, .2, .12, .06, .03, .02, .01 NC
 CONC OF ZINC BY AAS = 2142 PPM IN ASH

ELEMENT	ISOTOPE	CONC IN ASH PPM	CONC IN WET LIVER PPM
Ca	42.09	5413	85.53
V	51.06	8	.12
Cr	52.04	9	.14
Fe	54.25	26088	412.19
Mn	55.23	118	1.87
Co	59.09	9	.14
Cu	63.23	776	12.25
As	75	0	0
Se	80	16	.25
Rb	85.29	341	5.39
Mo	98.01	98	1.54
Ag	107.01	20	.32
Cd	112.03	139	2.18
Sn	118.04	47	.73
Sb	121	5	.08
Te	128	0	0
Ba	138	0	0
W	184	0	0
Au	197	0	0
Tl	205	0	0
Pb	208	16	.25
Bi	209	0	0
Th	232	0	0

Fig 3. A specimen output from the Autodensidater for the analysis of selected trace elements in liver tissue.

When the quantitative procedure has been fully developed it will be used to establish normal levels from a survey of selected human livers. This survey will provide the necessary background data for the interpretation of casework results when, at a later stage, the facility is made available to the regional laboratories.

Electrical detection is not currently used for normal project work but it is hoped to develop and utilise the equipment in a future project concerned with the examination of small fragments of metal.

The procedure for glass analysis has been extended to samples weighing as little as 100 - 300µg. Reasonable precision for important elements such as magnesium, iron, arsenic, manganese and barium has been obtained for these small samples. A survey of CRE results for 1mg samples, shows that modern window glass can be recognised with reasonable certainty using a combination of these elements. Results are recorded as relative exposures to calcium and these values are inversely proportional to concentration. Fig 4 provides merely one example of how magnesium, arsenic and barium ratios can be used to classify glasses. The cluster corresponding to modern window glass contains only two interfering samples, namely one container and a Japanese headlamp. The container could be distinguished when iron or manganese was used as one of the graphical axes but the headlamp was similar to modern window glass in all respects.

Similar graphical plots for classification are now being produced for 100 - 300µg samples. When these are completed glass fragments from normal clothing (CRE Report No 40) will be examined in order to determine what proportion could have originated from modern windows, a particularly important point in assessing the value of glass as evidence.

The spark source mass spectrometer has been used during the year to give assistance to regional laboratories in the analysis of glass fragments (1 case), small metal fragments (4 cases) and the analysis of toxic elements in tissue samples (3 cases).

B Glass, Paint and Fibrous Materials

Due to staff shortages during the year, paint and fibres have received only limited attention. The main emphasis has been placed on glass analysis.

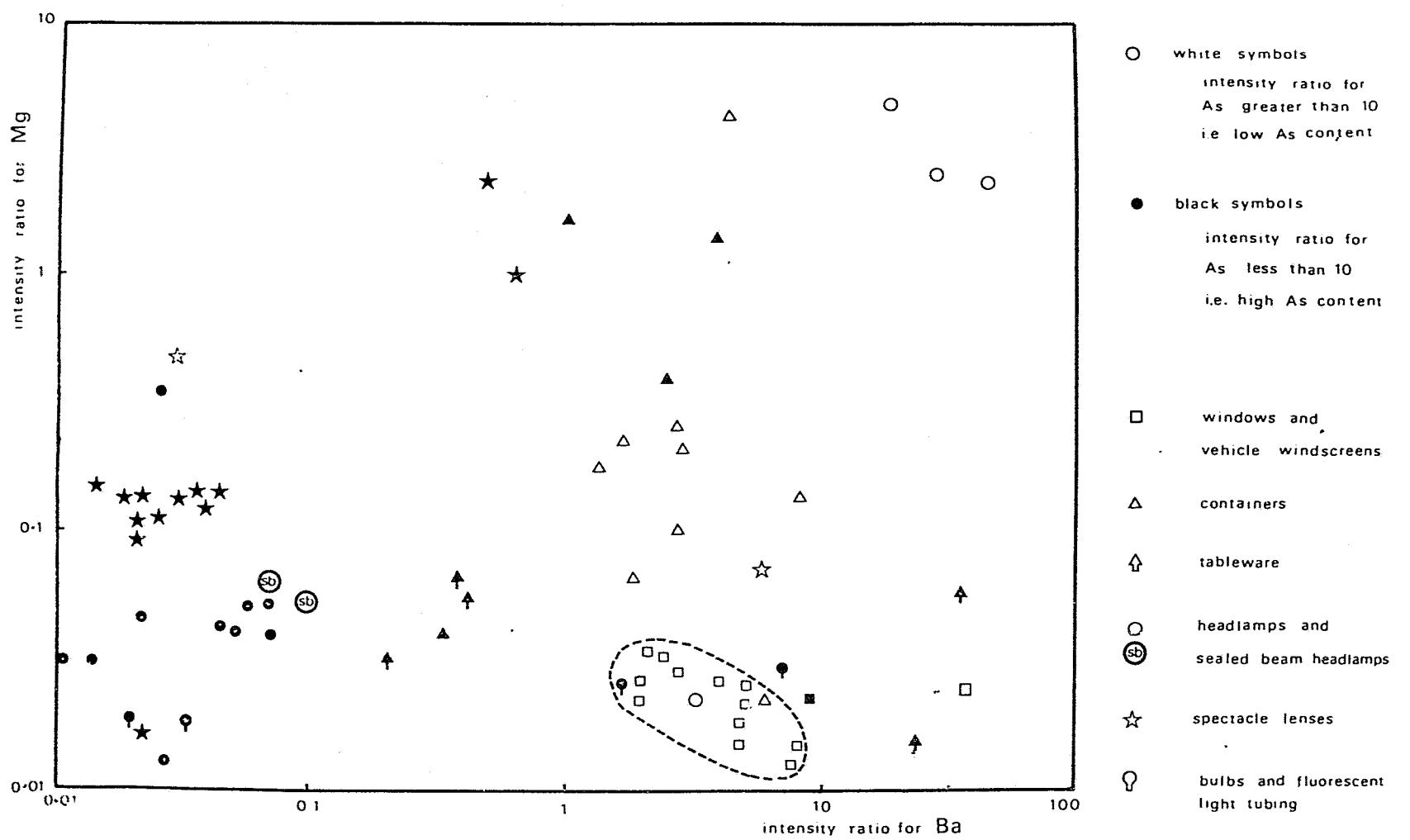


Fig 4. An example of how modern window glass can be recognised from Mg, As and Ba concentrations. The approximate boundary for modern window glass is shown as a dotted line.

(i) Glass

The automatic refractometer which was built by external contract was delivered at the end of June and is being evaluated. The instrument (Fig 5) is intended to measure the refractive index of 12 glass particles under automatic control. The samples are mounted in silicone oil and a cover slip is placed over the slide. The optical system is such that when the fluid and the glass have the same refractive index minimum light reaches the detector. At all temperatures away from the "match point" light is defracted by the glass and more light reaches the detector. The "match point" is detected electronically and the temperature printed out to 2 decimal places. Early results show that, although the detector system is working correctly, there may be a fundamental design problem. When the oil is placed in a multi-cavity slide and a cover slip placed on top it is almost certain that the cover slip will not be exactly parallel to the base of the slide. An angular error of a few milliradians is sufficient to produce inaccurate results. However if the system is manually compensated for imperfections in the cell then results are produced to an accuracy of better than $\pm 0.2^{\circ}\text{C}$ which represents a refractive index error of less than ± 0.0001 (See Fig 6). Manual compensations are not desirable for routine work and therefore alternative automatic solutions are being considered.

The Division continues to supply the service with calibrated silicone fluids for refractive index determinations as well as a standard sample of glass. A precision refractometer giving fifth decimal place accuracy is on order to make the calibration of the fluids easier and more reliable.

Flameless Atomic Absorption Spectrophotometry (FAAS) and Emission Spectroscopy (ES) have been used during the year for the determination of iron and magnesium in glass fragments. These methods are of particular interest because one and sometimes both techniques are already available in a regional laboratory. Iron and magnesium were chosen because they are useful elements for discriminating window from other sources of glass. A precision of approximately $\pm 10\%$ has been achieved for these two elements by FAAS on samples weighing $100\mu\text{g}$ (Report 176). The ES approach has been aimed initially at slightly larger samples and it appears that reasonable quantitation is possible using silicon as internal standard. The method can probably be extended to other elements and in any case

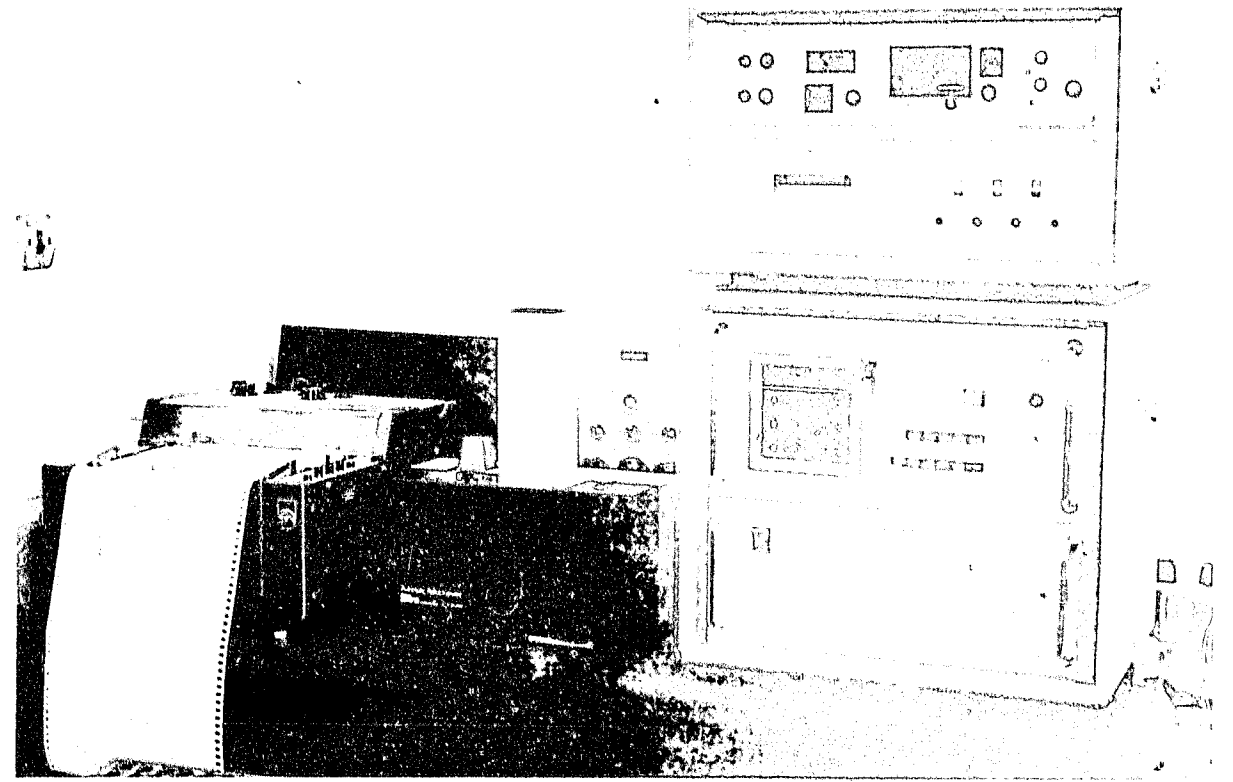


Fig 5. The automatic refractometer.

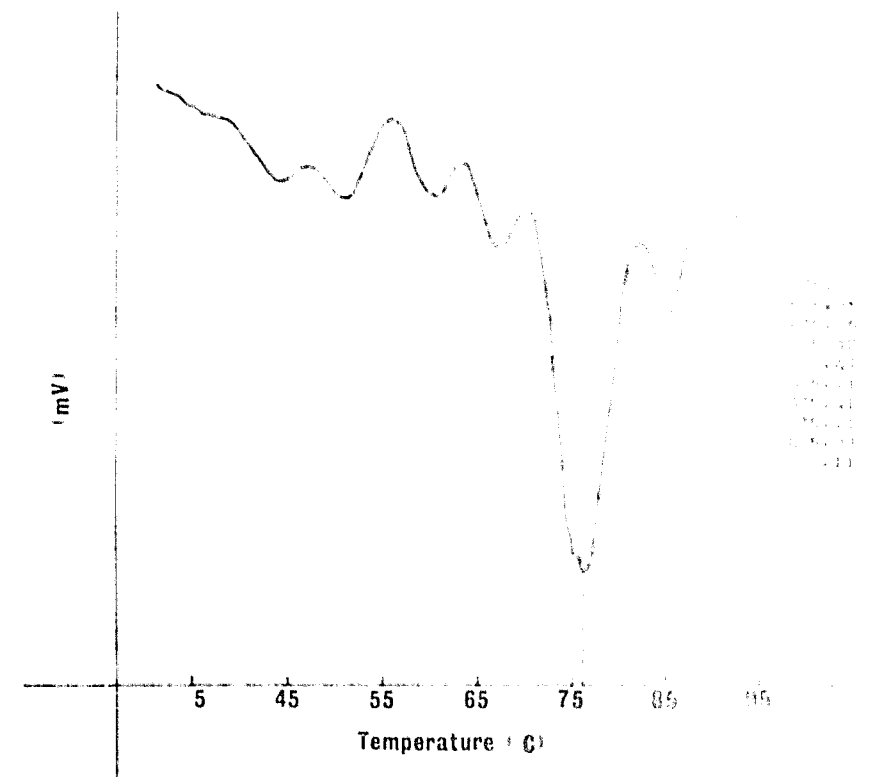


Fig 6. The change in output voltage from the automatic refractometer near the "match point". Match point temperatures for 10 fragments of glass from the same source (in a compensated cell) are shown.

a variety of elements can also be examined qualitatively. The use of an inductively coupled plasma source for emission spectroscopy is under active consideration. When appropriate analytical techniques suitable for use in regional laboratories have been developed they will be used to construct elemental distributions for a random collection of window glasses and for a collection of non-window glasses obtained from random breakages.

A survey of Energy Dispersive X-Ray Fluorescence equipment (XRF) has been undertaken during the year. The method has been shown to have great potential. Sub-milligram samples of glass were examined non-destructively by four manufacturers of the equipment and it was found possible to distinguish bottle glass, Pilkington (St Helens) window glass and Pilkington (Pontypool) window glass in a few minutes. We hope to obtain an instrument on loan early next year for a fuller evaluation.

(ii) Paint

A start has been made on the project concerned with the improved characterisation of multi-layered paint flakes. In the case of multi-layered white flakes from buildings, operators recorded 25% more layers by polarising microscopy than by simple binocular microscopy and the number of layers observed increased by a further 20% when U.V. fluorescence microscopy was used. As the samples used were taken from buildings the true number of layers present is of course unknown. Standard samples of known layer sequence are being prepared.

(iii) Fibrous Materials

A digest of all the information now available concerning trace elements in synthetic fibres is being prepared. The detection of these elements in casework samples provides many difficulties. Polyester samples have been melted on a graphite disc to form a small globule and examined using Proton Induced X-Ray Analysis (PIXA) in co-operation with AERE Harwell. Other possibilities include the examination of globules using XRF and the examination of ashed globules by Laser Emission Spectroscopy.

Four colour groups of fibres have been selected for a comparison of methods for dyestuff extraction and characterisation. Appropriate samples of these colours are now being collected.

Preliminary work suggests that zinc levels in hair are very constant over individual heads. The analysis by flame AA is quite rapid and may yield some useful information. Washing of the samples appears to be unnecessary.

(iv) Casework

The laser emission spectrograph has been used to assist regional laboratories in 4 cases and AA instruments have been used for quantitative analyses in 3 cases.

C Other Evidential Materials

(i) Gunshot Residues (Organic)

The major ingredient of modern propellants is nitrocellulose and this material has been detected on hands after single shots from a .22 semi-automatic pistol. However, the average amount recovered was only marginally greater than the detection limits of 2ng using TLC. It has not proved possible using the .22 calibre weapon to detect other residues, such as nitroglycerine which is present with nitrocellulose in some propellants.

It has proved possible to distinguish between nitrocelluloses of propellant and non-propellant origins using TLC, followed by Griess Reagent. Programmed Multiple Development was used in an effort to improve specificity and detection limits but without success (CRE Report No 167).

Because of an increase in terrorist activities, effort has recently been diverted from this project to the detection of explosive residues on hands.

(ii) Nitroglycerine residues on hands

Material for explosive analysis is typically removed from the hands of suspects using cotton wool moistened with solvent. Factors have been examined which affect the recovery efficiency at various stages in the pre-analytical treatment of hand swabs (CRE Report No 166). In particular the treatment of the swab was found to be critical at levels of nitroglycerine (NG) approaching the detection limits. About 5ng of NG was found to be lost immediately after allowing the swab to dry out and a subsequent slower loss occurred which was probably due to evaporation.

If a simple extract of the hand swab was used directly for analysis, skin oil contaminants were found to seriously reduce detector response in the gas chromatography (GC) for that and subsequent samples. A simple TLC clean-up procedure was developed to overcome this difficulty.

The gas chromatography of NG has been improved by using short small bore columns packed with a highly polar liquid phase. The detection limit for NG in processed hand swab extracts using these columns and an electron capture detector was 0.5ng. When the multiple ion detection system of the mass spectrometer was used as a GC detector, for ions at m/e 46 and 30, the detection limit was of the order of 2ng. A variety of detection reagents for NG were examined for use in TLC. The best reagent provides a detection limit of 2ng (CRE Report No 166).

Work on this project is continuing.

(iii) Soil

The soil project has proceeded rapidly during the last year and is now reaching a successful conclusion. The previous work concerned with dry, moist and ashed colour has been followed up by investigations of soil pH, saccharide content, cathodoluminescence and particle size distributions. Simple and rapid methods have been developed for the comparison of soil pH (CRE Report No 151) and total saccharide content (CRE Report No 168). Both procedures were reproducible down to small sample weights, yielded reasonable discrimination and were unaffected by storage for two weeks in sealed polythene bags.

The examination of sand fractions using cathodoluminescence was not particularly rewarding. The luminescence was dominated by either blue or orange, with relatively small amounts of red, green and yellow (CRE Report No 163). The discrimination offered was relatively poor and the results were not easily recorded. As a consequence this method was not recommended for routine use. However useful discrimination was achieved for ceramics and further work is being undertaken at Imperial College London, to determine the value of the cathodoluminescence spectra obtained from small glass fragments (CRE Report 163).

A Coulter Counter (Model ZB) (Fig 7) was purchased during the year and has been used to examine the size distribution of particles within the silt fraction (CRE Report No 152). The size distribution within the sand fraction has also been determined by sieving. The measurement of particle size distributions, within the silt and sand fractions, probably represents the most powerful approach so far examined for soil comparisons in forensic science. When the sand distributions of 18 soils were examined almost all pairs were clearly distinguishable using 1.5g samples. The considerable variation in silt distributions obtained using the Coulter Counter are shown in Fig 8 and these could be obtained using only 200mg of soil. A novel procedure for

the interpretation of particle size distributions is well advanced. The value of the various techniques, developed during this project, both alone and in combination has been reviewed (CRE Report No 164).

The only technique which remains to be evaluated is the use of density gradient columns. Bromoform/bromobenzene gradients have been found to be highly reproducible and their discriminating power for soils of similar dry colour is at present under investigation.

The various techniques developed have been applied to samples collected from simulated scenes of crime. Multiple control samples from small plots have been compared with soil removed from shoes, car tyres and plant roots. The aims of this work are twofold: to determine if the techniques are viable under casework conditions and also to recommend the best procedures for collecting representative control samples. Results to date appear very promising and suggest that all the methods are suitable for routine use.

It is hoped that in the next year the package of techniques, which have been developed and tested, will be directly evaluated by one or more regional laboratories in casework.

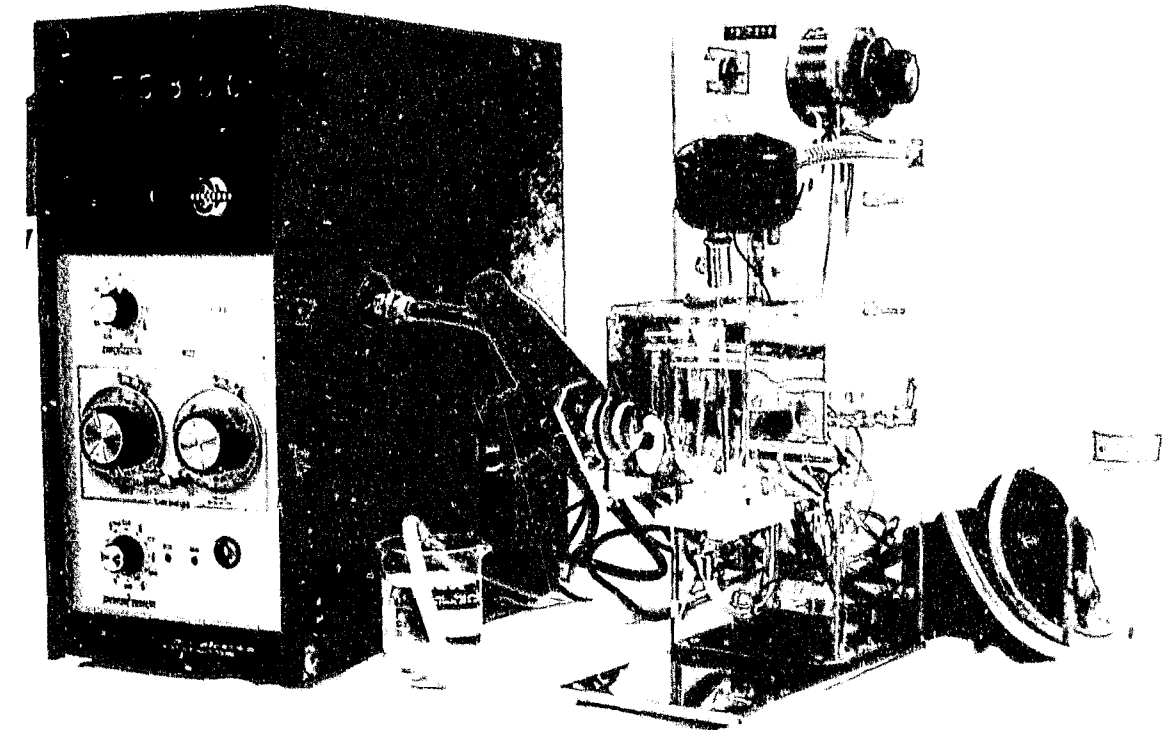


Fig 7. The Coulter Counter Model 2B.

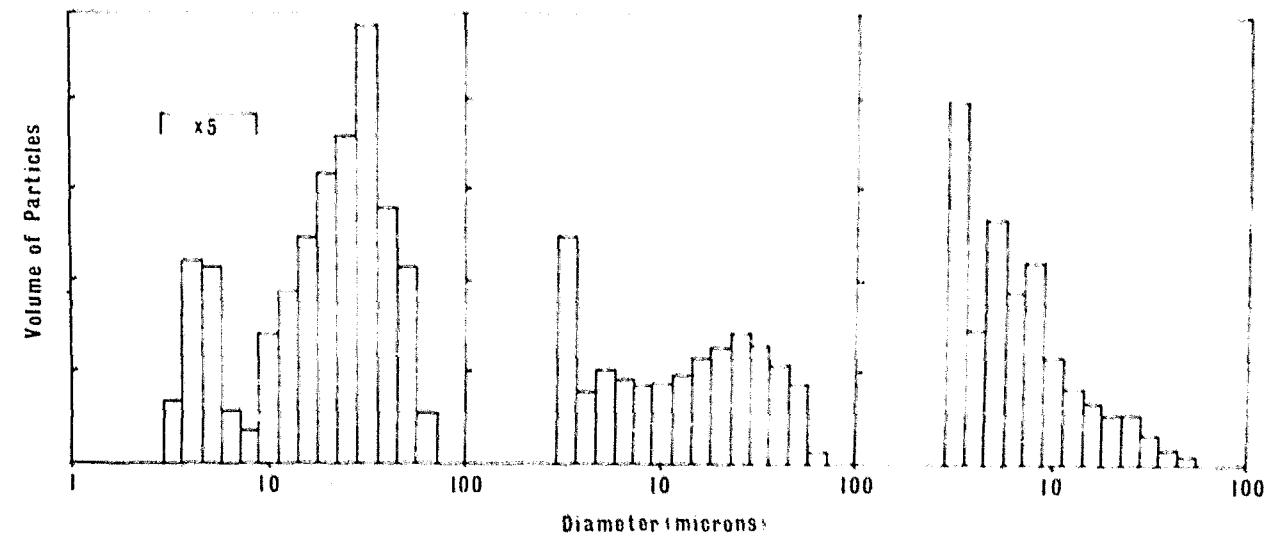


Fig 8. The results obtained from the Coulter Counter for 3 soils which showed marked differences in their silt particle distributions.

6. DRUGS DIVISION

(i) High Pressure Liquid Chromatography

An evaluation of various H.P.L.C. columns used for drug analysis is being carried out. So far, a reverse-phase (octadecylsilane) and a cation exchange column based on microparticulate silica have been systematically examined. Compounds chromatographed were selected as representative of a wide variety of drug substances of acidic, neutral and basic nature and of varying chemical structure, molecular weight, lipid solubility and pharmacological action. Both columns had the advantage that chromatographic retention could be varied in a highly predictable fashion. On the octadecylsilane column, elution was primarily dependent upon the pKa and lipid solubility of the drug and thus the eluent pH and methanol content were the major chromatographic variables. This reverse-phase column has proved most useful for the analysis of drugs and metabolites of neutral or acidic character, but for basic compounds the column efficiency was poor. (CRE Report No 153).

The microparticulate cation exchange column also operated in a predictable manner, based on a combination of reverse-phase partition and cation exchange chromatography. Thus, separations were influenced by the eluent, pH and organic solvent concentration (affecting the reverse-phase mode) and the eluent ionic strength (which affected the ion-exchange mode). As expected, for acidic drugs the column showed little selectivity, but for basic substances reasonable efficiencies were obtained (HETP=0.1mm). The restricted pH range and apparently short life of the cation exchange column detract from its overall usefulness. (CRE Report No 179).

The use of molecular filtration membranes for the removal of protein like material from biological samples prior to HPLC analysis has been examined. It was found that in some cases the irreversible adsorption of some drugs on the membrane could give very poor drug recoveries.

Investigations into the use of H.P.L.C. for the detection of drugs and metabolites in body fluids have continued and in this field the reverse-phase octadecylsilane system has several advantages. Not only can large quantities (up to 2ml) of aqueous sample (eg urine) be injected directly onto the column without adversely affecting the separation, but in the reverse-phase mode, the more polar metabolites are eluted before the parent drug. The use of the specific and highly sensitive fluorescence detector in such work is illustrated by

the detection of the metabolite of aspirin, salicylic acid, by the direct chromatography of only 5µl of urine, over a day after ingestion of a single therapeutic dose.

The work on the detection of LSD and its metabolites was hampered by the lack of authentic samples of the metabolites but Professor R T Williams and Mr Z Siddik of St Mary's Hospital Medical School London, have recently supplied samples and spectral data of two LSD metabolites, and these have proved extremely helpful. Case work involving the analysis of several body fluid samples suspected to contain LSD has been carried out. In one instance the presence of LSD in stomach washings at a level of 6ng/ml was indicated by the HPLC retention volume and by the fluorescence excitation and emission spectra of the eluted material. Although in this case radioimmunoassay gave an almost identical level of LSD, in a second sample involving urine which gave a positive radioimmunoassay, no unmetabolised LSD was detectable by HPLC. The use of HPLC in the investigation of cannabis metabolism is described elsewhere.

A number of cases from regional forensic science laboratories have been sent to CRE for analysis by HPLC. These include a case of suspected criminal poisoning of a child with aspirin, where samples of junior aspirin tablets, orange juice and stomach contents were analysed for acetylsalicylic acid, salicylic acid, saccharin and benzoic acid in an attempt to determine how the aspirin was administered. Using HPLC the direct qualitative and quantitative analysis of these four components was performed in four minutes without the need for any prior extraction procedure.

Chinese Heroin samples have also been analysed by HPLC and a case sample which was thought to include thebacon was shown in fact to contain acetylcodeine. The two compounds differ only in the position of a double bond and their mass spectra were very similar. This was a good example of the combined uses of mass spectrometry and an HPLC separation.

(ii) Blood Alcohol

Carbopack A coated with 0.4% Carbowax 1500 is now used by a number of laboratories but is no longer commercially available. A reserve stock of this material held at CRE was distributed to seven laboratories and this is sufficient for their current needs. A new phase Carbopack C coated with 0.2% Carbowax 1500 will be evaluated.

A polyurethane column which eluted alcohols in the order n-propanol, ethanol and methanol was examined for the analysis of ethanol in blood. (CRE Report No 175). This would have provided a completely different order of elution to the columns currently in use. Several columns were made and had efficiencies of 700-800 theoretical plates (measured with C₁₄H₃₀). Unfortunately broad tailing peaks for the alcohols were obtained necessitating an increase in sensitivity setting of x10 compared to the C₁₄H₃₀ peak. These results have been discussed with the authors of several papers who described the use of polyurethane columns and it appears that our results are typical of these materials. Coating the polyurethane with stationary phases such as Carbowax 400 or 1500 reduced the tailing of the peaks, but the order of elution of the compounds was then the same as that on conventional Carbowax columns and was therefore of no advantage.

Ten batches of breath testing devices have been tested during the last twelve months for their suitability for use under the RTA 1972. In May this year the Alcotest^R 80/A was approved by the Home Secretary for use by the police. This new device involves only minor changes, concerning the design of the mouthpiece, bag and container box, of the previously used Alcotest^R 80.

(iii) Radioimmunoassay

Increased casework and research involving radioimmunoassay (RIA) led to the purchase of an automatic gamma counter with 400 sample capacity in mid-1974 and an automatic diluter-dispenser in 1975.

The number of post mortem blood samples received for cardiac glycoside RIA in the last year was 21. Two of these were from suspected Lanatoside C overdose cases. Digoxin was detected in 17 samples and 10 of these contained toxic or lethal amounts. In 1975 the laboratory joined the newly formed Supra-regional Assay Service Digoxin Quality Control Scheme based at St Luke's Hospital, Guildford.

The cardiac glycoside assay service is to be taken over by the Aldermaston FSL in the near future.

Antisera to LSD-protein conjugates, which were raised in rabbits at the Microbiological Research Establishment last year, were found to contain significant anti-LSD antibody levels. The affinity of these antibodies was, however, too low for their use in RIA. A further attempt was made to raise suitable antibodies in sheep using the same LSD conjugates as before. After only 2 injections one sheep yielded a good antiserum. This was used at a dilution of 1:5000 in a conventional RIA system and gave a lower limit of detection of LSD of 10pg. However, urine contains an interfering substance which gives a background level equivalent

to 500pg LSD per ml. A disadvantage of this assay is the 3-day incubation period necessary for good sensitivity. A more convenient assay was developed by coupling a purified globulin fraction of the serum to solid Enzacryl-AA particles which are used in a solid-phase assay system with a total assay time of 3 hours. The lower limit of detection in this system is about 250pg LSD and normal urine does not then interfere significantly. The shelf life of the immobilized antibody, as presently prepared, is quite short (approximately 3 weeks) but work is in progress to improve coupling efficiency and develop this assay for routine use. The solid-phase antibody has also been used to recover LSD from urine prior to detection and measurement by high pressure liquid chromatography.

We are collaborating with Professor Harland's group at the Department of Forensic Medicine, University of Glasgow who have prepared an anti-LSD antiserum using a different conjugate to the one that was used to produce the antibodies at MRE. Their antibody and the CRE/MRE antibody are at present being evaluated using the same biological samples and the same analogues of LSD. It is hoped that the combined use of these two antisera will enable a highly specific LSD-RIA to be developed.

Several methods of extraction of insulin from human post mortem tissue have been investigated using radioiodinated insulin as a tracer. The most efficient method proved to be an acidified ethanol-water mixture. Losses of insulin by adsorption onto glass and kieselguhr, a very necessary filter aid, were greatly reduced by incorporation of 1% polyvinylpyrrolidone in the extractant. Recoveries from tissue doped with insulin were then in the 80-90% range.

Three commercial insulin RIA kits have been investigated and one of these, a solid phase system, has been used for most of the tissue work. Pharmaceutical insulin preparations contain insulin of bovine or porcine origin, both of which react similarly to human insulin. However, most of the preparations require treatment with 0.1M HCl to convert the insulin to an immunoassayable form. Tissue insulin concentrations can be measured by RIA after the initial extract has been converted to an aqueous solution and levels in several post mortem samples have been determined. The levels found in these were comparable with normal blood levels but fatty tissue had a higher content than muscle.

A single case of alleged attempted murder by insulin was investigated at CRE in the last year. Syringes, needles and insulin ampoules were received from the police and insulin was detected in all the exhibits. Help in two other cases involving the possibility of insulin overdosage was given to Forensic Science Laboratories.

(iv) Collection of drugs and analytical data

The collection of drugs numbered 981 a year ago which represented 860 different drugs and 121 different salts of the drugs. Determined efforts have been made to bring this collection up to date by including all those drugs in:

- A) In Professor E G C Clarke's book "Isolation and Identification of Drugs" Volumes 1 and 2
- B) All drugs mentioned in the Pharmaceutical Journal's Index of New Products from 12th August 1974
- C) Since 27th September 1975 the new products listed in the Pharmaceutical Journal

In addition, most of the compounds covered by the Misuse of Drugs Act, 1971 have been collected by Mr D Watson of Aston University under a contract with the Home Office and these are now in CRE's collection.

The collection contains 1383 drugs with a further 217 on order as on 1st November 1975.

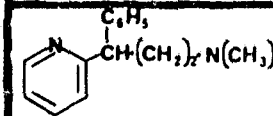
We are indebted to the many pharmaceutical manufacturers who have so generously given samples to this collection and also to the Metropolitan Police Laboratory, Professor A H Beckett (Chelsea College) and Dr S H Curry (London Hospital Medical College) for their donations.

The form of presentation of the analytical information has now changed from microfilm to hard copy and a specimen sheet is shown in Figure 9. The data sheet contains:-

- A) Drug name, chemical name, synonyms, proprietary preparations containing the drug, Wiswesser line notation, structural formula and molecular weight.
- B) Ultra-violet spectrum in 0.05M H₂SO₄ and strongly alkaline solution (The E_{1%} value is included)
1cm
- C) Infra red spectrum of the drug, with spectra of the drug salts on the reverse of the sheet

PHENIRAMINE

WLN: T6SJ BYR&2N1&1



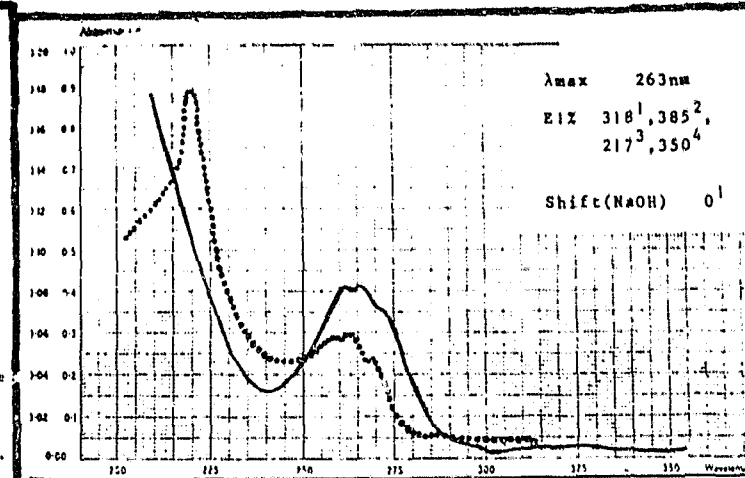
Synonym: Prophepyridamine

NN-Dimethyl-3-phenyl-3-pyrid-2-ylpropylamine

M. Formula $C_{16}H_{20}N_2$

M. Wt. 240.4

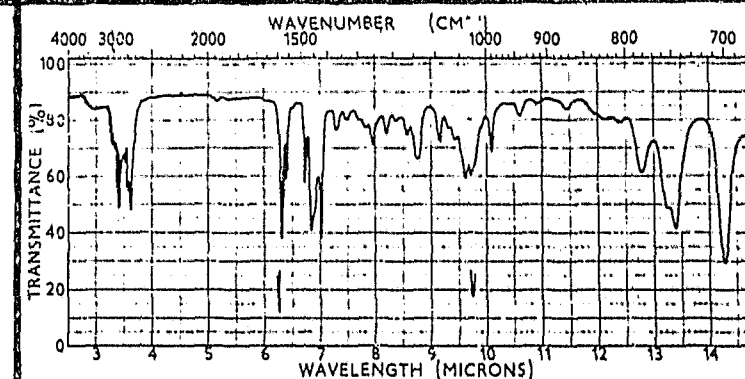
Proprietary names
Pheniramine Aminosallylate
Avil; Daneral.
Pheniramine Malate;
Inhiston;
Metron; Trimeton; Tripton.



CHROMATOGRAPHY

GLC 1805

TLC C:T:D 35
CHCL₃: MeOH 1:1
Acetone 3
UV light 254nm +ve
Fluorescence 254nm -ve
Ninhydrin -ve
FPN -ve
Dragendorff +ve
Iodoplatinate +ve
Marquis -ve



Liquid Film

NOTES

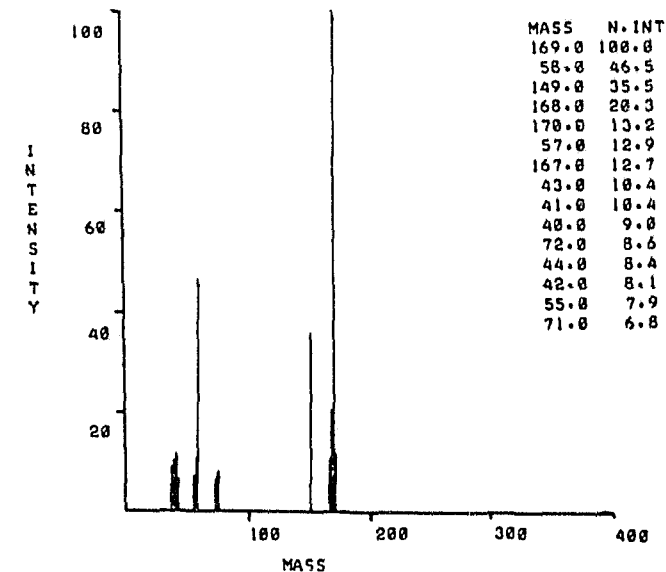


Fig 9. An example of the new drug data sheets

- D) Mass spectrum
- E) TLC data on three systems that have been standardised following combined trials with the Forensic Science Laboratories

They also include reactions of spots on TLC to 254nm ultra-violet light (both absorption and fluorescence) and to five spray reagents.
- F) GLC retention index values on SE-30 columns
- G) A space for Notes which can be used later for HPLC and CI-MS data
- (v) Analysis of blood and urine for cannabinoids

Professor V Marks and his associates at the University of Surrey have developed a RIA procedure for the detection of cannabinoids in body fluids where antisera were raised against a THC - bovine serum albumin conjugate. This antisera reacted with THC, some of its metabolites and metabolite conjugates. Urine samples from healthy volunteers gave blank values in the assay equivalent to less than 2ng THC/ml urine. Samples of urine from volunteers who have smoked cigarettes containing cannabis resin, gave results in the region 40ng - 1µg/ml.

The antiserum was obtained by CRE in June 1975 and an assay useable in the range 0.5 - 30ng THC/ml urine was developed using ³H - labelled THC. Work has so far confirmed the earlier findings regarding levels of cross-reacting material and attempts have been made to find other drugs which interfere in the assay. Thirty drugs so far tested did not react significantly.

In order to determine which of the metabolites of THC react in the RIA, a rabbit was given ¹⁴C - labelled THC intravenously and urine samples were taken. These samples were chromatographed using HPLC and the eluent was fractionated and measured for radioactivity. This enabled a radiochromatogram to be constructed which allowed the profile of drug metabolites in the urine to be established. Using aliquots of the eluent in the RIA procedure showed that metabolites of THC cross-reacted in the assay. Results so far demonstrate that all these metabolites are conjugated.

Work is also in progress to evaluate a GC/MS procedure for the identification and quantitation of THC in blood. Radioimmunoassay should give a reliable screening procedure for cannabinoids in body fluids whilst GC/MS

may provide a suitable confirmatory identification and quantitative estimation.

BIOCHEMICAL TOXICOLOGY

Within the last few months a biochemical toxicology group has been formed whose primary aims are:

- 1) to augment existing chemical methods of analysis with more sensitive or specific biochemical procedures for detecting toxic substances.
- 2) the identification of biochemical lesions in post mortem tissue caused by toxic amounts of drugs.
- 3) the interpretation of results following toxicological analysis in relation to the cause of death.

Work has commenced on an enzymatic assay method for the identification of fluoroacetamide, and fluoroacetic acid poisoning based on the use of a linked enzyme system involving aconitase and isocitrate dehydrogenase to monitor the presence of fluorocitric acid in post mortem tissue.

A gas chromatographic method has been set up for the determination of Kreb's Cycle acids (di- and tri-carboxylic acids found in tissues) which play an essential role in cell respiration. Methoximes are formed from any keto groups present and hydroxyl and carboxylic acid groups are trimethylsilylated. The relative concentrations of these acids will be measured in animal tissue at various times after death, both in normal and poisoned animals.

DRUGS INTELLIGENCE LABORATORY

The Drugs Intelligence Laboratory was set up in April 1975 at CRE on the recommendation of the Home Office Working Group on the application of scientific aids in the detection of drugs offences. The work of the laboratory has continued along similar lines to that undertaken in a successful operational research project. This was designed to look at the facility by which intelligence information obtained from seizures of illicit drugs could be exploited to the best advantage. The main objectives of the work have remained the same and the major functions are to:-

- 1 Collect, collate and disseminate information on new trends in drug abuse and
- 2 Examine in detail certain preparations of interest.

The existing liaison with the Central Drugs and Illegal Immigration Intelligence Unit and the Home Office Drugs Branch has been formalised and a regular bimonthly meeting is held to discuss topics of current interest. International contacts with America and Australia have been maintained throughout the year and are a feature of the information work. The laboratory has participated in the continuing work of the Home Office Working Group on the application of scientific aids in the detection of drugs offences. Resulting from the recommendation made this year an initial inquiry into the precursors used for illicit drug manufacture has commenced, in conjunction with the other agencies involved.

The practical aspect of the laboratory work has been extended, with the result that the development of new methods and techniques is a continuing project.

A seminar on "Drugs Intelligence" was held at CRE for the regional forensic science laboratories. The object was to provide a forum for the free exchange of views on the practical aspects of the Drugs

Intelligence Programme and an opportunity to hear about the work of the other government departments involved. A similar conference for the regional laboratory senior police liaison officers was held at the Central Drugs and Illegal Immigration Intelligence Unit. This was to acquaint them with the work of the Unit and the function of the Drugs Intelligence Laboratory. During the year lectures have been given at the National Drug Squad Conference and to regional police training departments on topics related to the work of the laboratory.

The bulletin "Drug Abuse Trends" for distribution to the regional forensic science laboratories and closely related organisations has been issued at approximately two monthly intervals. The last issue being No 14. October 1975.

The main drugs encountered this year, apart from Cannabis, have been LSD, Amphetamine, Methylamphetamine, Cocaine and Hash Oil. As a result of the increase in seizures of Hash Oil a comparative study of samples from cases throughout the United Kingdom has been started.

7. CONTRACTS DIVISION

The past year has been a very successful period for the Division culminating in the completion of twelve contracts. This is a direct consequence of the large number of contracts which were negotiated during 1971/73. A feature of the year has been the considerable amount of time spent by personnel in Contracts Division on the preliminary assessment and troubleshooting of equipment built under contract and it is becoming increasingly evident that, at least as far as contracts involving the construction of equipment are concerned; this is going to remain an important function of the Division.

Pyrolysis gas chromatography using a Curie point pyrolyser was established as a valuable technique for forensic scientists some years ago following a research project at CRE into the pyrolysis/gas chromatography of paints. Two contracts have been completed this year which extend our collection of pyrograms into the field of rubbers and fibres. The former is a collection of pyrograms of the basic raw rubber polymer, vulcanised rubbers, using various cure systems, and rubber blends. In addition various rubber products have been examined including tyre tread rubber and shoe soles. A large variety of both natural and synthetic rubbers and rubber-like materials have been examined including some of the more unusual rubbers. The contract involving the pyrolysis of fibres was a continuation of previous work, also carried out under contract, and was designed to update the present collection. New fibres are constantly appearing on the world market and it is necessary to have fresh contracts at fairly frequent intervals to ensure that the collection of pyrograms is maintained up to date. Both of these collections have been circulated to the regional laboratories.

As pointed out in last year's report, a collection of tyre tread patterns is envisaged which will contain the tread patterns of all the car and truck tyres available in the United Kingdom. In addition a coding system has been devised to distinguish between the various patterns and to assist caseworkers to readily identify a particular tread pattern from a track left at the scene of a crime. It proved difficult to devise a suitable coding system for all the tyres that are likely to be encountered and considerable effort has been put into the contract both by the contractor and

Contracts Division. However, a coding system has now been produced which will, on the basis of preliminary tests produce good discrimination between the various patterns. Delivery of the completed fully coded collection is expected early in 1976.

Negotiations are underway for work to be carried out on tyres relating to leakage rates and deflation damage. The programme of work on leakage rates will involve tests on the tyre in both static and dynamic states and the programme has been designed to clarify some of the remaining problems in this field. The research on deflation damage is being initiated because it is often useful to be able to estimate the distance a tyre has run in a deflated condition from the amount of damage that has been inflicted upon the tyre.

The problem of comparing paint colours has long been a problem in forensic science. Simple systems are already available which involve comparing the paint sample with a large number of standard colours. This system has the advantage of cheapness but obviously has its limitations. At the other extreme there are instruments available commercially which will compare paint colours automatically and give a read-out of the tristimulus colour co-ordinates. These instruments remove the subjectivity which exists in the simple system described above but are extremely expensive. A contract has been initiated for the construction of an instrument which steers a path between these two extremes. This microcolorimeter relies on a visual comparison of the paint sample with a standard and gives a read out of the tri-stimulus values. Hence using this method it is possible to make comparisons between similar paint samples and, more importantly, to obtain the tri-stimulus values, that is a numerical representation of the colour which can be stored in a retrieval system for reference purposes.

A contract has been initiated to find an additive that will prevent the clotting of blood in samples collected under the Road Traffic Act 1972. Initial results have been inconclusive but further work is planned and if this work is successful an additive will be available that will break down clots already formed in addition to preventing clot formation.

Two contracts for the construction of machines for automatically diluting blood with an internal standard solution are nearing completion. These instruments will remove samples of blood from the septum capped blood containers in current use and

dilute them into capped bottles ready for analysis. Two different models have been built and each has a number of safety features built in to ensure precision and absence of carry-over.

A commercially available automatic gas chromatograph using head-space analysis has been modified under contract to take a magazine of 200 samples rather than the 30 sample carrier available with the standard machine. A large number of teething troubles were encountered nearly all of which have now been solved. A complicating factor has been that it was difficult to establish whether the malfunction was occurring in either the new sample storage unit or in the commercially built section of the equipment. However, good progress has been made and the apparatus is now working reasonably well. (Fig 10). It is envisaged that this machine together with the automatic diluters should enable the Forensic Science Laboratories to cope with any likely increase in blood alcohol analyses under present legislation for many years to come.

The multi-element atomic absorption spectrometer has now been delivered to CRE. This machine was designed to utilize all the advantages of atomic absorption (speed, sensitivity, precision, freedom from interferences) with the multi-element capability usually associated with emission spectroscopy or spark source mass spectrometry. It was hoped that this instrument would analyse for 12 elements simultaneously with a sequential read-out from a modified Hilger and Watts direct reading detector. Although the idea is basically sound a great deal of practical difficulties were encountered not the least of which was the large amount of light loss which occurred because of the necessity to mount the single element lamps some distance from the collecting optics in order to accommodate the, relatively, large lamps. In use the instrument also produced some anomalous results which were difficult to explain. Whilst it may be possible to utilize this instrument as a research tool it is unlikely that it will ever become an easily operated routine instrument as originally envisaged.

The automatic refractometer (HORACE) for the measurement of refractive index of glass, which was designed at CRE and built under contract, has now been delivered and is being assessed. Although the electronics are working well the optical parts of the instrument appear to need further attention in order to obtain satisfactory results.

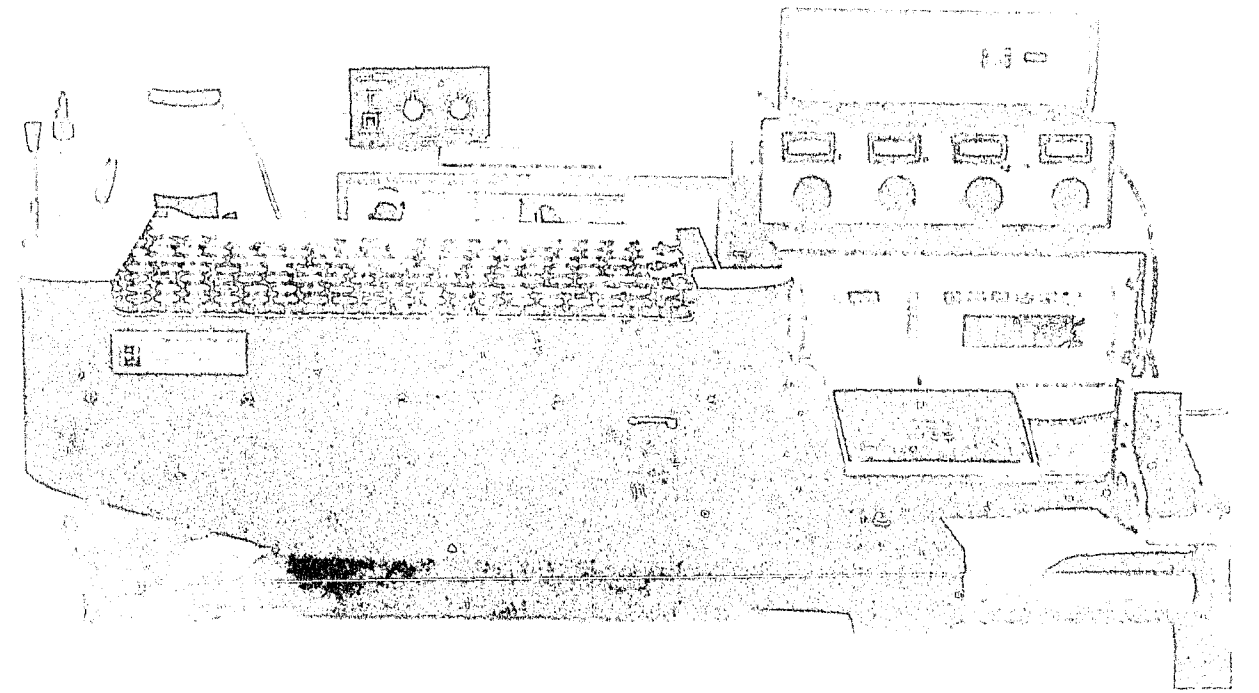


FIG. 1. A perspective view of the control panel and keyboard assembly.

Four toxicological contracts have been completed during the year. A report has now been received on the use of enzyme inhibition in the detection of trace amounts of drugs but, although one or two enzymes examined showed encouraging results the technique is unlikely to seriously challenge the more established methods of drug analysis in the foreseeable future. The determination of therapeutic amounts of antihistamines in blood has also been the subject of an external contract but unfortunately the work has been fraught with problems. Severe technical difficulties caused by the small amount of drug present were encountered in the early stages of the contract and later, difficulties with staff availability led to the contract's early termination. The final report, which could be specially valuable in connection with the problems which were encountered early in the contract, is awaited. A collection of analytical data on most of the drugs listed in the Misuse of Drugs Act 1971 has been received. The infra-red, ultra-violet and mass spectra of all these compounds together with TLC Rf's have been recorded and put on a computer file and a search program has been written enabling the file to be interrogated for one or more of the above characteristics.

An apparatus for the extraction of drugs from urine has been constructed under contract and has now been delivered to CRE. The apparatus uses 20 ml aliquots of urine which are extracted to give five fractions viz, morphine, basic alkaloids, strong acids, weak acids and neutrals. Quite a few teething troubles were encountered with this machine but all of these have been overcome except the problem which involves interface detection between the aqueous and non-aqueous layers. After extraction with ether the two layers are allowed to separate and then the lower layer is removed via an outlet in the base of the flask. It is important that as soon as all the lower layer is removed the outlet is closed and it is detection of this interface that is giving rise to problems. New detectors function quite well but an ageing effect appears to take place resulting in the interface detectors malfunctioning. The problem is being actively pursued at the present time.

At the moment in cases of death caused by drug overdose it is only possible to say that the deceased has taken a certain quantity of drugs on the basis of analysis. The ultimate objective of a contract on this subject is to be able to show how

a drug has acted on tissue cells to alter their performance hence causing death. As a first step the contract is designed to develop methods for the microscopic location of drugs in human tissue. A further contract in the field of drugs is designed to assist in the detection of drugs which are, for various reasons, normally difficult to detect in the human body. One possible solution to this problem is by an examination for the main metabolites of the drug. The ultimate object of the contract is to provide samples and data on the main metabolites of about two dozen named drugs which are common drugs of abuse and which cause particular difficulties in detection. A vast amount of data is published on drugs and their metabolites and most of the information which we require may already be available in the literature. Accordingly a contract has been initiated to carry out a comprehensive literature survey to find out exactly how much is known about the drugs in question. When this information has been accurately assessed we shall be able to precisely define what additional work needs to be done to obtain all the required data.

Two contracts have been completed resulting in the construction of equipment for the grouping of saliva samples. The first instrument was a continuous flow apparatus based on the Technicon system and was designed for the analysis of a large number of samples at a rate of between 25 and 50 samples per hour. The instrument is intended for fundamental studies on liquid saliva. The second saliva grouping machine is essentially an instrument for analysing batches of 10 samples at a rate of 6-8 samples per hour. The latter instrument works on the principle of haemagglutination inhibition and uses chromium - 51 labelled red-cells to quantitatively determine the amount of A, B and H blood group substance in saliva. Although the technique of using labelled red cells is well known it is understood that this is the first time that an automatic instrument using radio-actively labelled cells for grouping purposes has been constructed. Unfortunately considerable problems are occurring with this machine and work is continuing to try to obtain reliable results.

A simple low cost automatic saliva and blood grouping apparatus is being developed in conjunction with Biology Division. The object is to produce a simple piece of equipment costing only a few hundred pounds which could be used by the regional laboratories. The necessary equipment is being assembled at the moment and the laboratory prototype should be working early in 1976.

A contract has been initiated for the production of a quantity of anti-red cell lysate serum to assist in the work on blood grouping. Blood contains many different enzymes and current grouping techniques rely on the detection of each enzyme individually. Determining each enzyme separately can be time-consuming and expensive and the object of this contract is to raise an anti-sera against all proteins in human erythrocytes except haemoglobin. If successful such an anti-sera could lead to the development of techniques which would save considerable time in the characterisation of blood stains.

Also in the field of biology a study on the genetic basis of salivary proteins is about to commence. The study, which will use both liquid saliva and saliva stains is designed to discover the presence of further genetically determined protein variants. Variables such as storage and ageing will be investigated and studies will be made to elucidate the genetic basis of the protein under study.

In the last 2-3 years some interesting work has been carried out in Biology Division on the discrimination of blood stains on the basis of antibodies to disease organisms. Using just a few antibodies, discriminations of the same order as that obtained on the ABO system have been realised and the programme of research planned under this contract will extend the work into the field of viral antibodies. It is envisaged that with this technique discrimination should be greatly increased and techniques may be developed which will indicate the age of the individual who has spilt the blood and may also show whether he has travelled to various parts of the world. The vaccinations which prospective visitors are required to have by many countries before entry is allowed will have created many antibodies in their blood which should be detected by this technique. In addition a survey is being carried out of the types of antibodies found in a large number of blood stains.

The work on the investigation of protein levels in blood has been extended for a further year to consolidate the advances already made. A number of proteins have now been identified which may be useful in discriminating between blood stains.

The blood container project has been shelved but all the drawings and models have been retained so, if necessary, it can be re-started at short notice.

The cannabis detector, also mentioned in last years report, has now been exploited commercially and a company is now marketing it. The unit is completely self contained with its own power supply from rechargeable batteries. A small motor sucks air in through the inlet and deposits any entrained dust onto transparent adhesive tape. This tape is illuminated by a small lamp and viewed through a simple microscope mounted on top of the instrument. The instrument was designed as a screening device for use at airports etc and the initial reception of the instrument by possible users has been very good.

8. INFORMATION DIVISION

During the year part of the Division moved into the new Terrapin accommodation and this together with the reorganisation referred to in last year's report has enabled a more efficient and effective information service to be provided for the scientists in both this Establishment and the regional forensic science laboratories. Progress in the various areas of activity in 1975 is described in detail below.

A. Literature and Commercial Information

(i) Collection, Storage and Distribution of Scientific Literature

Papers for use by scientists in the regional forensic laboratories and also relevant to the current research programme of the Establishment are identified from the 60 journals including Chemical Abstracts and Current Contents (Life Sciences) taken by the Establishment, from the fortnightly biological and chemical profiles run on the United Kingdom Chemical Information Services (UKCIS) computer and from liaison with other establishments and organisations both in the UK and abroad. They are copied and passed either to the appropriate division in CRE or circulated to the regional laboratories and, in addition, these papers are allocated accession numbers, indexed in full, keyworded and microfilmed. By this process the Establishment's collection of papers are readily accessible using a computer retrieval program that has been previously described. Currently 17,200 papers of forensic interest are on file. During the year a programme has been initiated to evaluate whether the circulation to the regional laboratories in hard copy form meets the "customer" requirement by including a questionnaire with each months circulation. It is proposed to continue this until the end of the year and if required amend the current bias in the papers distributed.

(ii) Information Services

The number of enquiries from the regional laboratories dealt with has increased markedly during the year and now averages 100 per month. These typically involve about 30 using the computer either to trace papers on particular topics or to obtain some spectral or case frequency information. Another 40 are requests for specific papers and a further 20 are asking for some commercial or technical information that will often necessitate approaches to firms. The remainder are general information queries.

(iii) Communication Links

Following the trial reported in last years annual report of the Rank Xerox telecopier installed in CRE and at the Nottingham and Harrogate laboratories, experiments were carried out at the request of the Home Office Director of Telecommunications of the Plessey Remotecopier. This time the equipment was installed at CRE and at the Newcastle and Bristol laboratories. Taking into account the differences in size between these two laboratories and those involved in the earlier experiment a pattern emerged as to the likely usage of such equipment in the service and a report has been prepared.

The assessment of telex as a means of communication in the forensic science service continued with equipment installed at CRE, the Cardiff and Birmingham forensic science laboratories and at the Glasgow and Metropolitan Police Laboratories. This equipment was used much more than facsimile and it was felt that from the experience gained that there was a real need for telex in the forensic science service, for if it were generally available it would be widely used for communication between laboratories, the police and commercial organisations.

The experiments to assess the usefulness to the scientist in the regional laboratory of being on line with the CRE computer have continued with the co-operation of the Chorley and Newcastle laboratories and the Metropolitan Police Laboratory.

(iv) Inter-Laboratory Liaison

As last year, a programme was run with scientists from the regional laboratories attending CRE for a weeks attachment. In addition, a reverse attachment scheme was introduced whereby scientists from CRE spent a week in a regional laboratory. In order to improve the interchange of information between laboratories the information officers scheme was extended to include each laboratory and this has led to a major increase in information exchange and feedback.

B Collections of Scientific and Commercial Information

(i) Pharmaceutical Chemicals

The Division is now responsible for obtaining pharmaceutical chemicals from manufacturers for the forensic science service. During the year over 400 drugs have been received and the excellent co-operation of the manufacturers in responding is acknowledged. The Drugs Division are obtaining the IR, UV, mass spectra of these drugs together with other pertinent analytical data and this is being distributed to all regional laboratories in hard copy forms.

(ii) Soft Wood Collection

Photomicrographs illustrating the range of softwoods in the DSIR Bulletin No22 have been distributed to each regional laboratory.

(iii) PGC of Elastomers

The pyrograms of over 500 different elastomers prepared under an external contract with the Central Research and Development Division of Dunlop have been distributed in hard copy to the regional laboratories.

(iv) Headlamp Collection

The photographs and associated information on 361 headlamp lenses have already been distributed and it is hoped to distribute similar material on a further 130 shortly. With the increasing size of the collection a computer program has been written to enable a particular lens to be identified from part or all of the alphanumeric information on it.

(v) Shoeprints

Over 800 shoeprints plus associated information are now available in CRE. These have all been coded using the system developed by Dr A S Fawcett of the Nottingham Laboratory. An update is being prepared via an external contract with the Shoe and Allied Trades Association and when it has been finalised the complete collection will be microfilmed and distributed.

C Crime Scene Studies

(i) Fibres

The crime scene studies on the transference, persistence and mechanisms of fibre cross-transference and the efficiency of searching for fibres on clothing have been completed, and published (CRE Report Nos 126, 128, 134, 141).

(ii) Glass

The work previously started on the size distribution and numbers of fragments thrown backwards when 4mm thick windows of various sizes were broken was concluded. There was little difference between the fragment size distribution on the clothing of an individual standing in front of the window and that on the floor.

The persistence of glass on and in various articles of clothing and on the soles of shoes was also studied. On average after 40 days wear only 40% of the fragments initially present in pockets remained. For leather shoes only 3% of the fragments initially adhering to the soles remained after 20 minutes. With rubber soles a similar percentage remained after only 5 minutes. The rate of loss of glass fragments from 5 representative articles of outer clothing was surprisingly rapid with a finely textured sports jacket losing 100% in 3 minutes.

(iii) Case Involving the Analysis of Arsenic in Hair

During the year nine analyses for arsenic in hair were performed using neutron activation techniques. Four involved an incident in which four individuals were exposed to arsine gas. Samples of hair from all four were taken shortly after the exposure and at three further intervals thereafter. The results showed that in the event of an unknown exposure to arsine gas analysis of the hair would enable a realistic estimate of time of exposure to be made. Experiments are being carried out to monitor the level of arsenic in the head and beard hair of a volunteer who had ingested 6 milligrams of arsenious oxide. Analyses on the hair samples taken before and after the subject had undergone a period in a climatic chamber showed that the sweat contained low levels of arsenic (0.01ppm) and that it did not leach out the elevated level of arsenic in the root section to any significant extent. 2mm sectional analysis of the hair taken 44 days after ingestion showed that the position of the band of arsenic along the length of the hair was consistent with the expected growth rate of approximately one centimetre a month. (To be published).

D Collection of Information from Case Reports

This project, the background to which was described in some detail in last years annual report has continued and data from all the glass and tyre cases encountered in regional laboratories, has been recorded. Currently the number of records in each file are 4023 and 4285 respectively and interrogation of these files is providing the regional laboratory scientists with a ready assessment of the significances of this type of evidence.

Progress with the transition from feasibility experiments in other case type areas such as toxicology, paint, fibres etc to a live system has for a variety of reasons been slower than hoped. Advantage has been taken of this delay to examine the feasibility and practicality of replacing the forms with optical marked cards with the benefit of eliminating the data conversion step. Initial trials have been successful. Currently, versions of optical marked cards are before the toxicology, paint and glass Inter-Laboratory Advisory Committees and subject to their agreement data collection in this form can start.

(ii) Quality Control

Quality control experiments have continued during the year for the blood alcohol analyses, the grouping of blood and semen, the determination inorganic poisons in foodstuffs, the determination of glass refractive index and the identification of material on clothing. Because of the heavy involvement of the laboratories with alcohol analyses under the provisions of the Road Traffic Act 1972, aqueous alcohol standards are circulated weekly and blood alcohol standards fortnightly.

E Computer Services

(i) Organisation and Equipment

During the years the only change to the computer system has been the addition of a fast paper-tape punch which it is hoped will provide 'back-up' copies of programs for added security and additionally a convenient medium for information exchange.

(ii) Services of Information Division

(a) Information Room

The literature retrieval system is being maintained. Some preliminary work has been started on the documentation of the system for a possible commercially exploitable 'package' and on redesigning the format of the thesaurus. Customer requests have been made for an additional literature retrieval system based on bibliographic information and a pilot trial has been initiated to test the feasibility of storage, retrieval and indexing of papers in this way.

A short project on the feasibility of searching Chemical Abstracts in computer readable form was undertaken.

The results of the study were encouraging but the cost of the magnetic tapes containing the information made the system uneconomic for our purposes.

(b) Collections of information from case reports

The tyre retrieval system has been 'overhauled' and the documentation produced. A modification has been made to the glass summary program so that occurrences over a range of possible measurement errors can be produced. The first output of this form was produced in March. Progress in other areas has been slow and the transition beyond the feasibility studies for blood, fibres and general data has not yet been implemented.

(iii) Services to Chemistry Division

(a) MS Processing

During the year the programs for the production of bar-charts and peak-listings for mass spectra have been completed and several hundred mass spectra have been recorded.

(b) MS Search Indexing

The programs for the '8 peak' collection of spectra were written during the year and these provide the facility to search approximately 1000 spectra on file also to produce indexes (base peak, molecular weight, alphabetic). The indexes can be of the whole file or of the updates since the last full file listing (current awareness). No further work in this area is anticipated.

(c) Wiswesser Searching

A system to produce possible identities from structural information derived from spectroscopic evidence has been produced. Details of this system are reported in CRE Report No 157. The file stands at ~2500 compounds at present. An update of the system in the near future is envisaged.

(iv) Services to Other Divisions of CRE

At the end of the financial year several modifications were made to the computerised accounts system run for the administration section. This system is still under review and optical mark-card input will be introduced for the next financial year. A retrieval system for data on allergen tests was written for the biology division and this has been shown to be feasible but is still at a fairly early stage.

(v) Services to Regional Laboratories

Two data retrieval systems have been written during the year for particular regional laboratories.

a) The Birmingham Laboratory has been provided with a system for the rapid retrieval of typewriter typestyle information. This is used regularly and illustrates an invaluable use of telex,

b) A small trial system for X-ray diffraction data has been investigated for the Chorley Laboratory. Further additions have been made to the blood data retrieval file reported in the last annual report which now contains about 2500 records.

(vi) Colour Measurement

The initial assessment of the PRA fibre optics colorimeter has been completed and a Report is being prepared. Using ideal samples, ie standard colour cards, no difficulties were encountered with reproducibility and the machine was as good as the eye at distinguishing small colour differences. Accordingly it will be particularly valuable in collecting colour data for the statistical treatment of colour frequency information and programs have been written to assist with the calculation of colour differences. The reproducibility was less good with small case work samples (<1sq mm). This was attributed to colour variations, the presence of extraneous material etc, and the difficulty in placing these specimens evenly over the viewing aperture. A marked improvement was achieved by installing a light spring mechanism to the viewing head.

As part of the ongoing interest in measuring multi-layer samples edge on, several microcolorimeters have been examined with Contracts Division during the year.

(vii) Vehicle Paint Collections

Data on the topcoat and undercoats of new British cars and many foreign ones have circulated to the regional laboratories during the year and a start has been made on coding the comprehensive collection of paint panels held at Cardiff.

(viii) Photographic Services

The Division provides a comprehensive photographic service for the Establishment.

9. TOXICOLOGY DIVISION

The research effort of the division during 1975 was generally biased towards providing ready short-term help to the practising toxicologist in regional laboratories.

Work was continued on the isolation of difficult compounds, and on the action of solvents on basic drugs. In addition further studies on interfering compounds arising from putrefaction, and the testing of a prototype model of a time-saving automatic machine for extracting drugs from urine samples are being undertaken.

One change in the work in 1975 was the transfer of radioimmunoassay to the Drugs Division. Progress in the research projects is described in more detail below:

A Difficult Compounds

(i) Fluoroacetamide

This water-soluble compound is extracted by solvents from aqueous solutions when the latter are saturated by a salt. The method described in last year's Annual Report where the amide was extracted by methyl iso-butyl ketone (MIBK) from a salt-saturated acid solution was found to give low recoveries, possibly because of acid hydrolysis of the amide group during the concentration procedure. A modification of the original procedure was used in which the filtrates were prepared using sample-deproteination with a solution of aluminium chloride in 2N hydrochloric acid, and then saturated with potassium carbonate prior to dichloromethane extraction. This resulted in improved yields from spiked blood samples and higher tissue levels from the analysis of the viscera of poisoned animals (See below).

(ii) Fluoroacetic acid

Fluoroacetic acid is more volatile than the amide and losses from tissue extracts due to this property are likely to occur during concentration in a stream of air, even at room temperature. It was found that if a solution of fluoroacetic acid in MIBK was evaporated by blowing air over the surface of the liquid, the weight of acid left in the solution after concentration was only one seventh of that obtained when an equal sample was evaporated by bubbling air through the liquid.

Using the latter technique there was only 35 - 40% recovery indicating that the main loss in analysis occurs at the concentration stage.

In view of these findings the extraction procedure for the acid described in 1974 was modified.

(iii) Recovery of fluoroacetamide and fluoroacetic acid from spiked tissue slurries and tissue levels found in poisoned animals

To test the approximate recovery efficiency of each method, amounts of fluoroacetamide and fluoroacetic acid from 1µg to 20µg per g of tissue, were added to animal and human liver macerates which were then analysed as described. For the amide, recoveries fluctuated between 25% and 50% (one result only) with a mean at 30% and for the acid, between 25% and 80% (one result only) with a mean at 40%.

Estimations were based on the intensity of the most characteristic ion, FCH₂⁺ (m/e value of 33) which was measured by ion-monitoring the eluates from the GC column at the pre-determined retention times of the compounds.

Macerates prepared from liver, kidney, lung and heart tissues of the animals poisoned in 1974 were analysed and levels found (µg/g) are compared with those given in last year's Annual Report in the table below:

<u>Compound</u>	<u>µg/g found</u>		<u>1975 corrected for recovery</u>	<u>Remarks</u>
	<u>1974</u>	<u>1975</u>		
Fluoroacetamide	3	3	10	Lower volatility, no methylation required
Fluoroacetic acid	1-2.5	7-11.5	17-28	1974 extracts blown down 1975 extracts bubbled down

In view of the fluctuations in recovery of the compounds from spiked tissues some analyses on tissues of poisoned animals will be carried out by adding a measured quantity of the compound being sought to a half-portion of a tissue macerate. After the analysis of both half portions a more accurate figure for the recovery expected from the sample can be calculated.

Even with these precautions, volatilization during extract evaporation, and competition for methylation by co-extracted tissue acids are still factors which are likely to affect recoveries of fluoroacetic acid.

(iv) Sensitivity of detection of fluoroacetamide and fluoroacetic acid (methyl ester)

Solutions and extracts of known concentration were successively diluted and 1µl of each dilution injected into the GC column with ion monitoring for the diagnostic FCH₂⁺ ion. The minimum concentration of the amide and the methyl ester in the 1µl injection sample were respectively 0.1µg and 0.02µg. Using the recovery factors given, and assuming that 50µl of extract has been derived from 25g of viscera, this corresponds to tissue levels of 0.7µg/g fluoroacetamide and 0.1µg/g fluoroacetic acid.

These levels are well below reported LD₅₀ levels for fluoroacetamide in animals and man (1.5 - 2µg/g in rabbits, possibly 10-20µg/g in man) and for methyl fluoroacetate in farm animals and man (0.5-2µg/g in sheep, horse, swine, 2-5µg/g in man).

The work in sections A(i) to A(iv) is included in CRE Report No 172.

(v) Benzodiazepin drugs:

The isolation of this group of drugs has given rise to certain problems as the drugs themselves are hydrolyzed to various derivatives of benzophenone during hot acid tissue deproteination. Certain members can also appear as a second stage metabolite of another member, (eg oxazepam from diazepam) and the same benzophenone can be obtained from two members on hydrolysis, for instance both oxazepam and chlordiazepoxide give 2-amino-5-chlorobenzophenone (ACB). Extraction of the benzophenone following tissue hydrolysis has, therefore, a limited use in identifying the original drug present.

The frequency of occurrence of benzodiazepins in cases of drug intoxication is second only to barbiturates, and the start of what is envisaged to be a major effort has been made in this subject in 1975.

Direct solvent extraction of benzodiazepin drugs from blood, plasma or urine with organic solvents under mildly alkaline conditions gave a yield of 60% of added nitrazepam from urine, and 50% from blood samples.

With liver macerates the extracts were contaminated with interfering compounds, and it was essential to use an extract from a drug-free sample of the same liver (which is not possible in casework) to act as a reference in the UV assay. In addition, the liquid alkaline macerates emulsified with the extracting solvent owing to the high concentration of protein present. Deproteination of the sample under mild conditions using cold aluminium chloride solution (see section A(i)) yielded a filtrate from which no nitrazepam could be extracted.

These problems were to a certain extent, overcome by rendering blood and liver-water macerates alkaline with ammonia solution or sodium bicarbonate and adding anhydrous sodium sulphate to the mixture to form a damp solid "cake". The "cake" was then macerated with ether and the benzodiazepin drugs (with any other alkaloids present) extracted back into 2N sulphuric acid from the ether. The basic drugs were extracted back into fresh ether which was then evaporated to low volume and transferred to a silica TLC plate. After running in chloroform-ethanol (95:5) the plate was sprayed with potassium iodoplatinate and the various coloured bands produced were scraped off into separate tubes. The benzodiazepin drugs and other alkaloids were then recovered from the plate material by the method given in section C(i) of the Annual Report for 1974. Yields obtained from spiked samples were approximately 35-40% for nitrazepam, and 25-30% for chlordiazepoxide, diazepam and flurazepam. The UV assay of the last-named drug in the final dilute sulphuric acid extract had to be carried out within 5 minutes owing to the rapid hydrolysis which occurs.

Recovery of nitrazepam was highest (41%) from liver macerates of pH 9.5 and decreased with decreasing pH, being only 23% at pH 7.5. The drug was unstable in sodium hydroxide solution (0.5N), especially if warmed. Recoveries of nitrazepam and flurazepam by the ether extraction of ammoniacal, sodium chloride-saturated urine were respectively 75% and 95%.

It was found that no change in the initial concentration (20µg/ml) of flurazepam occurred after 6 weeks storage in urine at 4°C. With nitrazepam the initial concentration (8µg/ml) was maintained for the first 4 weeks but coinciding with a mould growth it was noted that a decrease of about 50% occurred over the next 4 weeks with a gradual distortion of the UV spectrum.

The TLC system used above to purify the ether extracts, separated benzodiazepin drugs from one another and also this class from other basic drugs such as dextropropoxyphene. Tyramine, a base commonly found in liver due to putrefaction, was retarded on the plate virtually at the starting line.

A reversed-phase system was developed to separate clonazepam from nitrazepam (a difficult pair) and consisted of paper which had been soaked in a 5% v/v solution of tributyrin in acetone and dried, run in a 10% v/v aqueous solution of formic acid. Rf values in this system were clonazepam 0.09, diazepam 0.42, nitrazepam 0.47, chlordiazepoxide 0.83, and flurazepam 0.91. Two case-livers, which contained benzodiazepin drugs, were extracted by the method described above. The extract from Liver I gave the UV spectrum of diazepam/oxazepam, and after acid hydrolysis the product was identified by GC as ACB, derived from diazepam metabolites, or from chlordiazepoxide. Chlordiazepoxide was eliminated by the UV spectrum of the extract. Similarly an extract of Liver II yielded the same UV spectrum as that of Liver I. After hydrolysis, GC indicated that the product was the methyl derivative of ACB (MACB) derived from diazepam or medazepam. The latter compound was rejected on the UV spectrum of the extract.

Addition of nitrazepam to an aliquot of Liver II resulted in this drug being extracted in 50% yield. The hydrolysed extract yielded a GC peak for MACB as before, and also a new peak for 2-amino-5-nitro benzophenone (ANB) which is the hydrolysis product of nitrazepam.

B Action of Solvents on Basic Drugs

(i) Halogenated Solvents

With reference to the findings reported in 1974, it was found that if amitriptyline in hydrochloric acid solution was made alkaline and shaken with chloroform, very little amitriptyline (often none at all) could be re-extracted from the chloroform into dilute sulphuric acid for UV assay. The phenomenon occurred if hydrobromic acid was substituted for hydrochloric acid but was not encountered if sulphuric acid replaced hydrochloric acid. Recoveries from aqueous solution varied between 0 and 20% using hydrochloric or hydrobromic acid and between 40 and 70% for sulphuric acid of the same strength. The difficulty is accentuated by the fact that protein precipitation requires halo-acids to obtain a reasonable release of alkaloids from tissue protein; sulphuric acid precipitation gives comparatively poor yields.

After amitriptyline in hydrochloric acid was made alkaline and shaken with chloroform, an iodoplatinate-positive spot of Rf value 0 was obtained when a small sample of the aqueous solution was run on a silica TLC plate in cyclohexane-toluene-diethylamine (75:15:10). This contrasted with an Rf value of 0.85 for amitriptyline. When the same aqueous solution in the presence of added bromothymol blue was extracted at pH 7 with dichloromethane the substance was extracted as a bromothymol blue complex. This complex, after treatment with alkali, dissociated on a cellulose TLC plate in the tetrahydrofuran-formic acid solvent used by Stevens and Moffat for quaternary ammonium drugs. The liberated base (Rf value 0) was extracted from the plate and subjected to mass-spectrometry. Unfortunately the only strong mass spectral line obtained was at m/e 58 (the group -CH₂-N(CH₃)₂). It is hoped to apply the technique of chemical ionization to resolve the constitution of this substance.

A salting in - salting out effect has also been observed during the extraction of amitriptyline from chloride-containing solutions. This was very marked with chloroform, and almost non-existent with ether extraction. Recoveries of amitriptyline were at least 25% lower from solutions containing high concentrations of salt (5.5M) than from solutions containing low to medium concentrations (<0.25 to 1.5M).

In digests deproteinated by hot 5-7N hydrochloric acid, the salt concentration produced by neutralizing the solution with caustic soda would be 3-4M, and in the "high concentration" category.

With ammonium chloride the same effect was noted but to a lesser degree. The reduction in recovery of amitriptyline from aqueous solutions by chloroform extraction in the presence of "high salt concentration" was only in the region of 10% below that obtained from a "low salt concentration" solution.

These observations suggest that this salting phenomenon may play an important part in reducing the yields of alkaloids from aqueous solution by chloroform extraction, and that neutralization of acid digests with ammonia instead of caustic alkali would assist in reducing this source of interference.

It is well known that samples of chloroform exposed to air and light results in the production of phosgene. Amitriptyline in chloroform was, however, found to be unaffected when a prepared solution of phosgene in toluene was added to it.

Similarly some dichlorocarbene derivatives of amitriptyline were synthesized, but they did not correspond with the product formed by the chloroform extraction of the drug from alkaline solutions.

(ii) Impure solvents

Diethyl ether frequently reacted with methadone resulting in low recoveries of methadone and the appearance of a second compound. Exposure of methadone to diethyl ether containing different amounts of peroxide showed that peroxides in the ether caused destruction of methadone. Ether stored in direct sunlight for nine days in the absence of ferrous sulphate generated sufficient peroxides to convert 40% of the methadone originally present into a second compound. The time needed for this reaction was approximately that taken for an ether extraction namely 4-5 minutes.

These results were readily reproduced by using ether in which the peroxide content had been increased by exposure to hydrogen peroxide. The product formed after reacting methadone with peroxide-containing ether was examined using thin layer chromatography, UV spectrophotometry, and gas chromatography (GC), followed by electron impact and chemical ionisation-mass spectrometry. The compound has been shown to be 1,5-dimethyl-3',3'-diphenyl-2-ethylidene-pyrrolidine, commonly referred to as a cyclic metabolite of methadone. Methadone has been reported to be difficult to detect in drug users, yet high levels of the cyclic compound suggest that methadone has been taken.

The results of the solvent experiments implicated that the detection of a cyclic metabolite of methadone may have been an artefact inherent in the extraction procedure. In view of the importance of such an implication, urine obtained from a patient receiving methadone was examined for the presence of the cyclic metabolite of this drug using Amberlite XAD 2 resin and carrying out extractions of the urine with solvents other than ether.

Control urine samples with and without added methadone were also examined by the same procedures. The results showed that (1) the cyclic compound was produced and excreted as a urinary metabolite following the administration of methadone (2) provided that peroxide-free ether was used for extraction, no

artefactual results were obtained (3) chloroform was found to be as efficient as ether for extracting methadone and its cyclic metabolite from urine, and without the risk of peroxide-induced synthesis of the latter compound from the parent drug.

C Interfering acidic compounds of putrefaction

The aim of this investigation is the extraction and identification of interfering acidic substances from both fresh and putrefied livers. An atlas of properties of 40 acidic and 2 neutral compounds, that have been reported to occur as interfering factors in visceral extracts, is currently being compiled.

The ultra-violet (UV) spectrophotometry of these compounds has been completed and the $E_{1\%}^{1\text{cm}}$ values of the acids were recorded in 0.5N sodium hydroxide. Peak shifts between pH 12 and pH 2 were recorded. For neutral compounds the $E_{1\%}^{1\text{cm}}$ values were calculated in ethanol and spectra in 0.5N sodium hydroxide with peak shifts between pH 12 and 2 have also been recorded. The compounds have been run in two T.L.C. systems, one on silica gel plates using a chloroform-n-butanol-acetic acid mixture and the other on cellulose with n-butyl acetate-water-acetic acid and Rf values in both systems determined.

Location of the spots on the T.L.C. plates was carried out by the following methods:-

1. For both systems, absorbance or fluorescence in U.V. light (λ 254nm, 350nm);
2. For the cellulose plates only, the use of a bromocresol green spray and also a mixed fluorescein - ferric chloride spray;
3. For the silica plates only, the detecting reagents were acidic potassium permanganate and also a mixture of indicators with alkaline permanganate. As both solvent systems included acetic acid it was necessary to remove this from the plate, after running, by heating it in an oven prior to the application of any spray which contained indicators.

Acid and neutral fractions in ether, derived from tungstic acid deproteinated liver filtrates, have been run successfully in both T.L.C. systems and a number of ultra-violet absorbing spots noted.

A selection of barbiturates, which are the most commonly occurring acidic drugs in poisoning, was run in both systems. No separation occurred between barbiturates in either system. They ran as one spot free from all 40 putrefactive acids in the silica system, and from 39 out of the 40 in the cellulose system. This

indicated that viscera could be easily screened for barbiturates as a group in the presence of all the putrefactive acids examined.

A G.C. system for separating the putrefactive acids as their methyl esters is now in progress using an SE30 column.

High pressure liquid chromatography (HPLC) has also been successfully tried for separating mixtures of some of the acids. It is hoped that further work with this method will be continued, as the individual acids can be collected as fractions as they are eluted off the column.

Further work for the completion of the atlas of chemical properties of these compounds will involve compiling data on colour tests and mass-spectrometry.

At present some exploratory studies are being carried out on some old case-liver extracts.

Apart from the commonly occurring p-hydroxy-phenylacetic acid, a number of yellow-coloured compounds, thought to be similar in nature to the hydroxychalcones reported by Fox, Scaplehorn and Tonge have been isolated. It is hoped to examine these yellow compounds by mass-spectrometry.

A brief survey of the techniques used by the individual regional forensic science laboratories to extract acidic drugs from viscera has been made, and it is hoped that the information kindly supplied by these laboratories will be used with the findings of this study to ascertain the extent of the analytical interference caused at various stages of visceral putrefaction.

D

(i) Protein precipitation and alkaloid recovery

To complement the work reported in 1973, the analysis of liver tissue containing 10-20 $\mu\text{g/g}$ of twenty different alkaloids was investigated. The liver level chosen represented the lower end of the range 5-200 $\mu\text{g/g}$ reported in the case-samples from regional forensic science laboratories. Four deproteinating reagents, which are currently used in the regional laboratories, were used on liver-water macerates to which a measured quantity of each drug had been added. The reagents studied were (a) sodium tungstate-sodium bisulphate at 90° (Valov) (b) ammonium sulphate-hydrochloric acid at 60° (Nickolls) (c) 5-7N

hydrochloric acid at 90° (Dubost) (d)
Aluminium chloride in 2N hydrochloric acid
at 60° (Stevens).

The alkaloids were extracted from the filtrates
for assay as described in the 1973 Annual Report.

(ii) Assay Methods:

Liver, unlike blood, causes problems in the UV
assay of alkaloids in the 230-285nm region because
of interfering compounds which are co-extracted. An
extract from a drug-free sample of the same liver
has to be used as a reference solution in the
spectrophotometer to cancel this interference.
For this reason GC (SE30 column) was the method
usually employed, but in certain cases where difficulties
arose other methods were used. These included
high pressure liquid chromatography (HPLC) for
morphine and strychnine on micro-bondapak and partisil
columns respectively, spectrofluorimetry for quinine
(λ max, ex 350nm, λ max, em 450nm) and spectrophotometry
in the visible region of the spectrum (505 and
528nm) for phenothiazine drugs after oxidation with
perchloric acid - sulphuric acid.

(iii) Results

The results obtained paralleled those from the
blood recovery experiments of 1973. The Dubost
method gave the highest yields (60-80%) but
hydrolysed labile alkaloids like cocaine and
orphenadrine. Aluminium chloride deproteinization gave
lower recoveries (40-60%) but possessed the advantage
that none of the alkaloids under test were
hydrolysed. The tungstate and ammonium sulphate
procedures generally gave very poor results. Ten
nil recoveries were recorded for the tungstate method
and eight for ammonium sulphate, and these included
six alkaloids where recoveries were nil using both
methods.

With the exception of the Dubost method, phenothiazines
were recovered as their sulphoxides.

Water-washing the solvent extracts prior to extraction
of alkaloids into 2N acid caused apparent low recoveries,
due to loss of alkaloids (up to 47%) into the
wash. In order of decreasing magnitude of loss
the order was:

Amphetamine > Pethidine > Methaqualone > Chloroquine >
Nicotine and Quinine > Methadone and Perphenazine > the
remaining alkaloids which were unaffected by water-
washing.

Prolonged contact (48 hours) between added
alkaloid and visceral macerate caused no
significant difference in the recoveries obtained
for amitriptyline, chloroquine, cyclizine and
trifluoperazine.

From the findings described in this section, a
screening procedure for alkaloids is advised.
This involves aluminium chloride deproteinization,
followed by ether extraction from the alkaline
filtrate (prior to chloroform extraction) and drying
the solvent extracts with anhydrous sodium sulphate,
before finally back-extracting the alkaloids into
2N sulphuric acid.

The work described in section D has been circulated
as CRE Report No 165.

E Anti-asthmatic drugs

These drugs are difficult to extract from body
tissues for identification and assay, because they
are water soluble, and, being phenolic amines,
unstable in alkaline solution. Blood levels can
be less than 1 μ g/ml. Fatalities are alleged to have
occurred as the result of excessive use of certain
anti-asthmatic aerosol sprays containing the drugs
but the analysis of the post-mortem viscera has
proved very difficult.

Some initial work on the fluorimetric detection of
isoprenaline derivatives, and the separation of
trimethylsilyl (TMS) derivatives of iso- and orci-
prenaline and salbutamol was reported in the 1974
CRE Annual Report. During 1975 an effort has been
made to find an efficient method to extract these
compounds from aqueous solutions.

An ion-pair extraction method using bromothymol blue
(BTB) in phosphate-buffered solution at pH 7 enabled
the three anti-asthmatic drugs to be extracted into
dichloromethane.

The dried residues from the evaporated extracts were
heated with acetonitrile and trimethylsilyl imidazole
which converted the extracted BTB complexes
to TMS derivatives. The latter were then assayed
by GC-MS. Initially, these drugs have been
detected by the above method at levels in the
aqueous solution down to 1 μ g/ml and it is hoped to
achieve detection at a lower level than this.

Future research will involve extraction from visceral
samples with the concurrent problem of metabolism.

F Automatic urine extractor ("AUPEX")

This equipment was constructed under contract as a result of the experience obtained at CRE during 1967-9 when a bench prototype was built to study the problems involved in the automatic extraction of drugs from visceral filtrates.

The equipment has been under continuous test since April 1975 using aqueous solutions and urine samples containing added drugs.

When fully operational the results obtained were very satisfactory. Each class of drug tested was extracted into the correct one of five different fractions (strong and weak acids, neutral, alkaloid and morphine).

Recoveries were, in general, marginally below those obtained by manual extraction as is illustrated by results from a typical mixture in urine as follows:-

	<u>%Manual</u>	<u>%AUPEX</u>
Salicylic Acid	62	58
Amylobarbitone	53	51
Phenacetin	18	12
Nicotine	95	87
Morphine	51	25

The carry-over of drugs from one sample to another was very small when the machine was washed out automatically between analyses. (2-9µg of nicotine were carried over from 3480µg).

The time for the production of all five fractions was 16 minutes with about 8 minutes for washing out, which was considerably faster than using a manual method.

The precision and reproducibility of analyses on identical samples was good when the equipment was fully functional. However many small electrical and mechanical faults developed, most of which were rectified during the test-period, but the chief remaining source of trouble centres around interface detection between organic solvents and aqueous solutions.

A number of differently-styled detectors have been tried but all have suffered so far from deterioration with use. They gave high initial performances but these fell off until nil responses were obtained during the analytical process.

It will be necessary to ascertain the reasons for this deterioration, and to design a more reliable detector for use in future operational work.

APPENDIX A

LIST OF STAFF MEMBERS

<u>Name and Division</u>	<u>Rank</u>	<u>Telephone Extensions</u>
DIRECTOR		
Dr A S Curry	DCSO	5853, 5856 4212
(Mrs P J Tew, Personal Secretary, 5853, 4212)		
DEPUTY DIRECTOR		
Mr G W Walker	SPSO	5853, 5856 4212
(Mrs P J Tew, Personal Secretary, 5853, 4212)		
BIOLOGY DIVISION		
Dr P H Whitehead	PSO	5947
Mr E R Rutter	PSO	5938
Mr J Sutton	SSO	6273
Mr M Davie	SSO	5937
Dr A E Kipps	SSO	6273
Dr P E Burdett	HSO	5937
Dr D J Werrett	HSO	5937
Miss V E Quarmby	SO	6273
Mrs M J Dorrill	SO	5937
Miss C M Bosley	ASO	5937
Mr R Burgess	ASO	5937
Dr L A King	SRF	6273
Dr J M Twibell	SRF	5933
CHEMISTRY DIVISION		
Mr K W Smalldon	PSO	5947
Mr B German	SSO	5505
Dr R Dudley	SSO	6279
Dr J Locke	SSO	7574
Dr J D Twibell	SSO	6279
Dr R E Ardrey	HSO	5505
Dr R Macrae	HSO	6275
Mrs I B Beattie	SO	5505
Mr C R Howden	SO	5505
Mrs D Morgans	SO	7574
Miss J M Home	ASO	6279

<u>Name and Division</u>	<u>Rank</u>	<u>Telephone Extensions</u>
DRUGS OF ABUSE		
Dr A C Moffat	PSO	5947
Dr P Twitchett	SSO	6394
Dr S Fletcher	HSO	6275
Mr P L Williams	HSO	6394
Dr A T Sullivan	SRF	5938
EXTERNAL CONTRACTS DIVISION		
Dr M D G Dabbs	PSO	5930
Dr R Holleyhead	SSO	5930
Mr D S Loxley	HSO	5930
INFORMATION DIVISION		
Dr A Scaplehorn	PSO	6951
Mr C Brown	SSO	5952
Mr M Swain	SSO	6996
Mr C A Pounds	SSO	4289
Mr A R Allan	HSO	6996
Mr G Owen	HSO	4289
Mr J Porter	HSO	5952
Mr E M Besly	SO	6996
Mr M Harold	SO	6996
Mr B C Platt	ASO	4289
TOXICOLOGY DIVISION		
Dr H Stevens	PSO	5947
Dr M D Osselton	HSO	5938
Mr P Owen	HSO	5933
Miss T Holdstock	SO	5938
Mr M D Hammond	SO	5938
DRUGS INTELLIGENCE		
Mr P J Gomm	SSO	5948
Mr I J Humphreys	HSO	5948

<u>Name and Division</u>	<u>Rank</u>	<u>Telephone Extensions</u>
TECHNICAL STAFF		
Mr D J Nicholson	P & TO III	5783
ADMIN STAFF		
Miss M North	EO	5502
Mr S Jones	CO	5942
Mrs P Ridout	CO	5560
Miss A Livingstone	Typist	5853
Miss A Mair	Typist	5560
Miss P Hale	Trainee Typist	5924
Miss H K Payne	CA	5942
Mrs P J Tew	PS	5853
NON-TECHNICAL STAFF		
Mrs M M E Chapman	Cleaner	6631
Mr D Gabb	Lab Att.	6631
Mr L Rowbottom	Driver	6631
SANDWICH COURSE STUDENTS		
Miss M A Burkett		6273
Mr G G F Cadwallader		5938

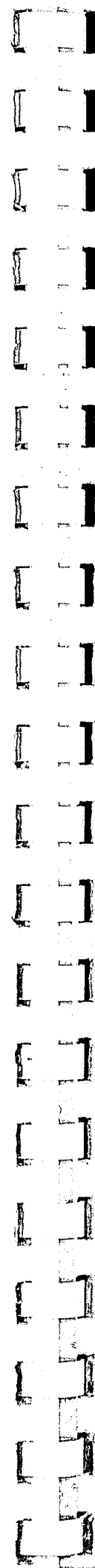
APPENDIX B
CURRENT PROJECTS

BIOLOGY DIVISION

Head of Division - Dr P H Whitehead

<u>Title</u>	<u>Progress</u>
DISCRIMINATION STUDIES USING NON-GENETIC PARAMETERS	
Blood	
Drugs	No other drugs detected in micro bloodstains since salicylate identified (CRE Rpt. 107). Feasibility study on metabolites of nicotine initiated.
'Antibody Profiling'	Techniques now established and show considerable long term potential for gaining information from blood- stains relating to donors age, race, clinical history (including allergy status) and environment. Operational 'Blind Trials' initiated. (CRE Rpts. 154, 161, 162).
Clinical Biochemical Parameters	Completed. Limited value in Biochemical Profiling at present (CRE Rpt. 108).
DISCRIMINATION STUDIES USING GENETIC PARAMETERS	
Semen	
Acid phosphatase	Seminal AP distinguished from vaginal AP (CRE Rpt. 150). "Blind" Trial on swabs in progress in collaboration with Chorley Laboratory.

<u>Title</u>	<u>Progress</u>
Other semen enzymes and proteins	Quantitative studies on PGM levels in semen suggest present electrophoretic techniques inadequate for low PGM levels (CRE Rpt. 173).
	Quantitative studies on levels and stability of PGM in semen and semen stains initiated.
Saliva	
Amylase genetic variants	Rapid technique established for detecting amylase variants (CRE Rpt. 149). Further work in contracts.
Other salivary enzymes	To be started.
Other genetic markers	Inv and Gm markers in saliva and semen detected, which offers new means of discrimination (CRE Rpt. 155).
Blood	
Studies on established enzyme Variants	PGM typing of blood simplified by use of "Ready Mixed" Reagents (CRE Rpt. 143) Anti-sera to red-cell enzymes being raised by external contract.
Laurell electrophoresis	Protein work by external contract.
Immunology Studies	
Use of Latex:	Batches of latex evaluated and supplied to regional laboratories. Preparation on a larger scale planned by external contract.
Species identification of blood and tissue	
Serology	Application to ABO antigens not yet successful.
Other antigens	To be started.



<u>Title</u>	<u>Progress</u>
General Studies	Amylase sensitive paper developed previously has shown wide distribution of saliva on 'normal clothes' (CRE Rpt. 156). The effect of this saliva background on routine bloodstain grouping is under investigation.
<u>Correlation Studies</u>	Studies into the correlation between enzymes and serological groups started. Original observations made on amylase levels in lip secretions. (CRE Rpt. 127). Saliva-semen correlation studies dependent on supply of samples.
<u>Automation</u>	
Saliva	Technicon auto-analyzer for grouping in A, B & O systems simultaneously on liquid saliva in operation. 'Fiori's concept' not confirmed. Correlation studies on saliva-semen inter-relationships initiated.
<u>Hair</u>	
Sheath Cells	Problems encountered with sexing of sheath cells. Microscopic studies on frequency of occurrence of sheath cells show traditional views need revision (CRE Rpt. 171).
Keratin Studies	Initiated.

CHEMISTRY DIVISION

Head of Division - Mr K W Smalldon

<u>Title</u>	<u>Progress</u>
MASS SPECTROMETRY	
<u>Organic (MM 16F)</u>	Upgraded Instrument recently installed. New applications of MS in forensic chemistry being sought.
Service facility for other divisions	Project co-operation with Toxicology and Drugs Divisions. (Report No 172).
Services for regional laboratories	Collections of normalised spectra for common drugs, 8 peak index search system for 1,000 organic compounds, Wiswesser structural retrieval system for 2,500 compounds and commercial Mass Spectral Search System for 31,000 compounds already available. Collections being continuously updated from the whole of the forensic science service. (Report Nos 157, 159, 169, 170, 174).
<u>Inorganic (MS 702)</u>	
Trace elements in liver tissue	Analysis of liver ash for more than 40 elements shown to be feasible. Quantitative SSMS method and complementary AA methods being developed. Survey of normal livers to follow. Low temperature asher on loan from Harrogate laboratory (Report 177).

<u>Title</u>	<u>Progress</u>
Analysis of small glass fragments	Method now developed for analysis of Mg, Mn As and Fe in 100-300µg glass fragments. To be used for the trace element analysis of glass fragments from normal clothing (See Glass, Paint and Fibres Project below)
Service facility for regional laboratories	Analysis of glass and metal fragments in appropriate cases. Service for trace elements in liver tissue to be offered later.
GLASS, PAINT AND FIBROUS MATERIALS	
<u>Glass</u>	
Atomic Absorption	Method now developed for Fe and Mg in 100µg samples using non-flame AA. Data now required to aid interpretation of these trace element concentrations. (Report 176).
Emission Spectroscopy	Quantitative method for specific trace elements being developed. Other elements to be checked qualitatively.
Analysis of glass fragments from normal clothing	Trace element analysis on glass fragments retrieved from normal clothing using a combination of AA, Emission Spec. and SSMS. Elements being sought which best distinguish window glass controls from normal clothing glass.

<u>Title</u>	<u>Progress</u>
Automatic Refractometer	Instrument now delivered from external contract. Designed to measure RI of 12 glass fragments automatically. Evaluation in progress.
<u>Paint</u>	
Smears	Characterisation of smears to be investigated. Methods used to include laser emission spectroscopy.
Difficult multi-layer systems	Methods of characterising difficult multi-layer flakes, such as multi-layer whites, are being investigated using intact flakes. Values of binocular, polarising, UV fluorescence and cathodoluminescence microscopy already being compared. Etching of binder to expose pigment particles, followed by staining and luminescence microscopy to be investigated.
<u>Fibrous materials</u>	
Tracers and catalysts in synthetic fibres	Data collection concerning trace elements in polyester and acrylic fibres completed. Methods of analyses sought which could detect these differences on casework samples
Fibre dyestuffs	Various methods of dyestuff extraction and characterisation to be examined in an attempt to produce a recommended procedure. Groups of fibre samples of similar colour are now being collected.

CONTINUED

1 OF 2

<u>Title</u>	<u>Progress</u>
Hair	Elements which could be easily determined and yield at least some reliable information have been considered. Zn, Cu and Ca are preliminary selection. Zn variation over individual heads in pilot study <10% by flame AA.
Instrumental services for regional laboratories	Use of atomic absorption and laser emission in appropriate cases.
<u>Other Evidential Materials</u>	
Firearms blowback residues on hands	Detection limits for possible organic residues investigated. Hand swabs from firings examined but results so far unreliable. A wider range of weapons to be examined later.
Nitroglycerine residues on hands	TLC, GC and GC-MS methods examined and best detection limits obtained. Persistence of NG on hands being investigated. Handling experiments and persistence of NG on inanimate objects to follow. (Report Nos 166, 167).
Soil	Dry colour, ashed colour, pH, total saccharide content, cathodoluminescence and particle size distributions of soils now fully examined. Techniques developed being tested using samples from simulated scenes of crime. (Report Nos 151, 152, 163, 164, 168).

DRUGS DIVISION

Head of Division - Dr A C Moffat

Title

Progress

High Pressure Liquid

Chromatography

Evaluation

Both cation exchange and octadecylsilane columns have been evaluated for a wide range of drugs. (Report Nos 153, 179).

LSD

LSD can be detected fluorimetrically in biological samples at levels of 5ng/ml. Retention data for analogues have been compiled. The separation of metabolites is in progress and identification by mass spectrometry should follow.

Case Work

The analysis of a number of samples for drugs of abuse and also biological samples for poisons has been carried out for forensic science laboratories

Blood alcohol

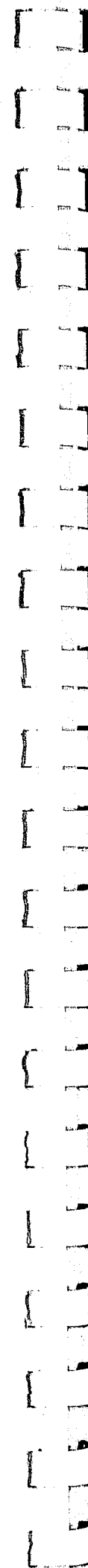
Assessment of containers

The vials used to contain blood for purposes under the RTA have been examined.

Improvement of analytical procedures

Polyurethane columns for blood alcohol analyses were found to be unsuitable for routine use. On-going commitment to increase efficiency. (Report No 175).

<u>Title</u>	<u>Progress</u>
Breath testing devices	Ten batches of breath testing devices have been examined. On going commitment.
<u>Radioimmunoassay</u>	
LSD	Antibodies to LSD from sheep are being used to develop an assay suitable for use with blood and urine.
Insulin	Methods of assay have been compared for the assay of insulin in human tissues and pharmaceutical preparations. Background levels of insulin in tissues will be determined.
Immunoassays	Conversion of RIA's to non-radioactive methods.
Case Work	Analysis of samples for LSD, insulin and cardiac glycosides has been a large proportion of the work. On-going commitment.
<u>Collection of Drugs and Analytical Data</u>	
Drug substances	1383 available.
TLC	The first 100 data sheets are nearing completion. The systems to be used have been evaluated.
GLC	
UV	
IR	
MS	



<u>Title</u>	<u>Progress</u>
<u>Analyses of blood and urine for Cannabinoids</u>	An electron capture GC method was found to be insufficient for the task. A radioimmunoassay procedure is being evaluated. Metabolites are being characterised by HPLC, RIA and MS after the administration of radio-labelled THC to rabbits. It is hoped to develop a method for the quantitative measurement of cannabinoids by GLC-MS.
<u>Biochemical Toxicology</u>	
Krebs Cycle Acids	Analysis of pure acids from aqueous solutions accomplished by ion exchange chromatography, freeze drying and GLC after derivative formation. Animals will be poisoned with specific respiratory poisons as well as CNS stimulants and depressants to see if any differences are apparent.
Investigation of the changes in tissue biochemistry following death and after the administration of toxic agents	To be started.
Development of <u>in vitro</u> biochemical methods for drug detection	A start on the analysis for fluoroacetate in body tissues using aconitase, has commenced.

INFORMATION DIVISION

Head of Division - Dr A Scaplehorn

<u>Title</u>	<u>Progress</u>
Collection, Storage and Distribution of Literature and Commercial Information	
In house searching of journals and abstracts	17200 reprints on HP2100A file, further papers being accessed
Use of UKCIS	Chemistry and Biology profiles run fortnightly
Literature presentation to regional laboratories	Selected papers to all laboratories monthly. Relevance study in progress.
Microfilm and microfiche	16,000 papers on film, CRE reports to 158 on microfiche
Preparation of forensic science bibliography for commercial exploitation	Index of authors, titles and journals in preparation
Communication Units	Facsimile trial completed and report written. Telex and direct computer link under trial
Collecting firearm data	Feasibility study started.
Collections of scientific and commercial information	
IR collection	New presentation of analytical data being undertaken in conjunction with Drugs Division
UV collection	
Collection of pharmaceuticals	
Register of human toxicology	Complete to end of 1974. Circulated in June 1975.

<u>Title</u>	<u>Progress</u>
Headlamps	Almost 500 lamps collected. Photographs of 361 lamps in laboratories. Data computerized.
Footwear patterns	Total collection of almost 1000 prints now coded.
Tyre patterns	Subject of an external contract.
Car paints	Top and undercoat colours for all British cars being computerized. Undercoat data for foreign models being collected.
House paints	Collection of household paint colour data to be started.
Analysis of arsenic in hair by NAA	Analytical service. Work on arsenic uptake by hair has started.
<u>Computer Services</u>	
Computer Services for Information Division	Computerisation of scientific, commercial literature and case return information. Investigating feasibility of recording further information.
Computer services for Chemistry Division	Off-line MS processing. Searching and indexing the MS collection. Structural retrieval.
Computer services for Administration Section	Accounts operational
Evaluation of the PRA colorimeter	Initial evaluation complete. Report in preparation.
Computer services for Regional Laboratories	Type style data and blood group data retrieval systems for Birmingham Laboratory.
Future computer needs of the Forensic Science Service	Appraisal commencing.

<u>Title</u>	<u>Progress</u>
Collections of Information from Case Reports. Quality Control and Photographic Services.	
Collection of Information from Case Reports.	Collection of casework data to help operational scientists with evidence presentation. Continual liaison with the Inter-Laboratory Advisory Committees. Tyre and glass forms continuing, blood, fibre, paint and toxicology data collections to start.
Analysis of Information from Case project reports.	
Organization of Quality Control results	
Analysis of Quality Control results	Regular commitment
Photographic Services	Service to all Divisions.

TOXICOLOGY DIVISION

HEAD OF DIVISION - Dr H M Stevens

<u>Title</u>	<u>Progress</u>
Fluoroacetamide/ Fluoroacetate detection	Complete except for reproducibility studies on analysis of tissues (Report No 172)
Protein precipitation/ drug release	Studies complete on blood and liver using chemical methods. Enzyme hydrolysis to be tried for acid-labile drugs
Extraction of benzodiazepin drugs from tissue	Basic method worked out for some common benzodiazepins. Will be coupled to benzodiazepin hydrolysis methods
Extraction procedures and assays for catecholamine drugs in tissues	Some work on assays by GC and fluorimetry of derivatives done. Investigations into extraction methods proceeding
Extraction of alkaloids from TLC plates for mass spectrometry with a study of ageing on plates	Completed (Report No 158)
Action of solvents on basic drugs	Characterization of product between amitriptyline and chloroform to be accomplished prior to other alkaloids being studied. Methadone - peroxidised ether product identified. Study continuing
Action of formal-saline on barbiturate levels in case-liver samples	Not started, awaiting staff

REPORTS PUBLISHED SINCE NOVEMBER 1974

<u>Title</u>	<u>Progress</u>		<u>Report No</u>	<u>Title</u>	<u>Date</u>
Acid compounds of putrefaction of human viscera	UV spectra, TLC of 40 control samples determined. Livers being analysed and endogenous acid compounds being examined				
Cyclic AMP in urine	All practical work done. Ph.D. thesis to be completed.		144	Studies on Saliva, Part V. A Comparison of Two Methods for the Detection of Saliva Stains.	November 1974
Use of selected enzymes to detect certain poisons	The action of fluoroacetic and fluorocitric acid in inhibiting aconitase is currently being investigated		145	The Assay of Amylase Using Amylase Sensitive Paper.	January 1975
Stability of drugs to storage in urine and viscera, especially when putrefaction occurs	Not started		146	Commercially Available anti-Rabbit and anti-Cow Sera for Species Identification.	January 1975
Survey of interfering substances in commercial solvents used for drug-extraction	Not started		147	A Technique for the Physical Examination of Illicitly made Tablets.	February 1975
			148	Studies on Saliva Part VI. Thiocyanate in Saliva - An Attempt to Discriminate Between Smokers and Non-Smokers.	February 1975
			149	A Rapid Technique for the Detection of Amylase Isoenzymes using an Enzyme Sensitive "Test Paper".	February 1975
			150	Iso-Electric Focusing of Seminal, Faecal and Vaginal Acid Phosphatase.	February 1975
			151	A Simple Method for Determining the pH of Small Soil Samples and its Use in Forensic Science.	March 1975
			152	Preliminary Evaluation of the Coulter Counter for Particle Size Analysis of Soils	March 1975
			153	High Pressure Liquid Chromatography of Drugs: an Evaluation of an Octadecylsilane Stationary Phase.	March 1975
			154	The Differentiation of an Adult's Bloodstain from that of a Child. Report of an inter-laboratory Blind Trial.	April 1975

<u>Report No</u>	<u>Title</u>	<u>Date</u>
167	Programmed Multiple Development - A short Evaluation of the Regis Chemical Co Instrument.	August 1975
168	A Colourimetric Method for the Determination of soil Saccharide Content and its Application in Forensic Science.	August 1975
169	Molecular Weight Listing of the CRE Structural Retrieval Computer File.	September 1975
170	Organic Mass Spectrometry. IV. Mass Spectrometry Comparison Trial.	September 1975
171	The Significance of Sheath Cells on Plucked Hair Roots.	October 1975
172	The Rapid Identification and Quantitation of Fluoroacetamide and Fluoroacetic Acid in Biological Materials.	October 1975
173	Optimal Conditions for the Assay of Human Seminal Plasma Phosphoglucomutase.	December 1975
174	Mass Spectra of some Benzodiazepines, their Benzophenones and Metabolites.	November 1975
175	Open Pore Polyurethane GLC Columns for the Analysis of Ethanol in Blood.	October 1975
176	The Determination of Iron and Magnesium in Small Glass Fragments Using Flameless Atomic Absorption Spectrophotometry.	December 1975

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155	Inv Grouping of Saliva and Semen.	April 1975
156	Studies on Saliva Part III. A Preliminary Survey of Clothing for Saliva Stains.	April 1975
157	Structure Retrieval using the Wiswesser Line Notation.	May 1975
158	The Recovery of Alkaloids after their Location on Thin-Layer Chromatograms	May 1975
159	Organic Mass Spectrometry III Forensic Science Mass Spectra Collection.	July 1975
160	Identification of the Major Excipients in Illicit Tablets using Infra-Red Spectroscopy.	June 1975
161	A Note on Anti-microbial Antibodies in Menstrual Blood (Vaginal Secretions).	July 1975
162	The Detection of Allergy-Associated Antibodies in Experimentally Prepared Bloodstains.	July 1975
163	The Construction and Applications of a Cathodoluminescence Microscope in Forensic Science.	July 1975
164	Techniques for Soil Comparison in Forensic Science.	September 1975
165	An Assessment of the Efficiencies of Four Protein Precipitation Methods used in the Extraction of Basic Drugs from Human Tissues.	September 1975
166	The Extraction and Identification of Nitroglycerine from Hand Swabs.	August 1975

<u>Report</u>	<u>Title</u>	<u>Date</u>		
<u>No</u>				
177	The Determination of Trace Elements in Liver Tissue by Spark Source Mass Spectrometry: Part I, Feasibility Study.	October 1975		A Radioimmunoassay Technique for Digoxin in Post-Mortem Blood. Phillips, A P; J Forens Sci Soc 1974, 14, 900.
178	An Improved Paper for Saliva Stain Screening.	October 1975		A method for Quantitating Amylase and its use in the Investigation of Various Body Fluids. Kipps, A E and Whitehead, P H; Anal Clin Biochem 1974, 11, 219.
179	High Pressure Liquid Chromatography of Drugs: II, An Evaluation of a Microparticulate Cation Exchange Column.	October 1975		Interpretation of Post Mortem Serum Levels of Cardiac Glycosides After Suspected Overdosage. Moffat, A C; Acta Pharmacol et Toxicol 1974, 35, 386.
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Km(1) (Inv(1)) Typing of Saliva and Semen.
Davie, M J and Kipps, A E; Vox Sanguin.

APPENDIX D

COLLOQUIA

The Colloquia are now a regular feature of CRE's activities. They are held in the winter months and attendances of nearly 200 scientists are not unusual. Details are given below of the 35 papers presented this year. In addition two Seminars have been held on Mass Spectrometry and Drugs Intelligence.

These meetings not only provide a forum for the exchange of scientific information, but in addition they are an excellent means of meeting colleagues in the Service.

We are grateful to the Recreational Society and the Atomic Weapons Research Establishment for the loan of the Sir William Penney Theatre.

'Colloquium on Toxicology'

Friday, 13 December, 1974

Chairman: Dr A S Curry

The Determination of Benzodiazepines as Benzophenones
P G Ashton - Forensic Science Laboratory
Aldermaston.

Discussion

The analysis of hair samples in a case of arsenic poisoning
C A Pounds - Home Office Central Research
Establishment.

Discussion

Inorganic Poisons - The Poor Relations
Miss J Wiles - Metropolitan Police Laboratory.

Discussion

The use of organic mass spectrometry in Toxicology case
work
Dr H M Stevens and Dr J Drayton - Home Office
Central Research Establishment.

Discussion

"Retrieval of the Analytical Data of Drugs of Abuse"
D Watson - Aston University.

Discussion

A case of Paraquat Poisoning
Mr M R Loveland - Forensic Science Laboratory
Birmingham.

Discussion

An Oenanthotoxin Poisoning
Mr M J Lewis - Forensic Science Laboratory Chorley.

Discussion

'Colloquium on Biology'

Friday, 10 January, 1975

Chairman: Mr E G Davies

Antibody Profiling of Blood Stains

Dr L A King - Home Office Central Research
Establishment.

Discussion

Interpretation of Blood Group Results and Errors in the
Presentation of Evidence

Mr J Craddock - Nottingham Forensic Science
Laboratory.

Discussion

The Selection of Blood Group Systems in Casework

Dr N Weston - Birmingham Forensic Science
Laboratory.

Discussion

The Incidence, level and Nature of Micro-Biological
Contamination of Blood Submitted for Ethanol Analysis

Dr Janet Corry - Metropolitan Police Laboratory.

Discussion

Evidence Relating to Blood Stain Distribution

Mr R Outteridge - Harrogate Forensic Science
Laboratory.

Discussion

The Preparation of Hair Replicas

Mr J Hancock - Birmingham Forensic Science
Laboratory.

Discussion

'Lewis Groups'

Mr P Martin - Metropolitan Police Laboratory.

Discussion

Observations on the Form of Group Specific Substances
in Aqueous Blood Stain Extracts

Mr M Davie and Dr P Whitehead - Home Office
Central Research Establishment.

Discussion

'Colloquium on
New Techniques in Chemistry'

Friday, 28 February, 1975

Chairman: P G W Cobb Esq

The Examination of Vehicle Paints by SEM/Electron
Microprobe

Mr J M Dubery - Birmingham Forensic Science
Laboratory.

Quantitative Emission Spectrography in the
Characterisation of Glass

Miss E Blacklock - Metropolitan Police Laboratory.

Compound Identification using Organic Mass Spectrometry
data

- (i) Instrumentation and Data Collections
Dr J V Drayton (CRE)
- (ii) Manipulation and Searching of Data using a
Mini-Computer
Mr C Brown (CRE).
- (iii) Retrieval using Structural Information
Dr R E Ardrey (CRE).

Selective G C Detectors in Drug Analysis

Mr A Clatworthy - Metropolitan Police Laboratory.

The Measurement of Density by a Magnetic Method

Dr D C J Horncastle - Bristol Forensic Science
Laboratory.

'Colloquium on
Training and Communication'

Friday, 7 November, 1975

Chairman: Dr A S Curry

Chairmans introductory remarks

Scientific staff training in Chorley Forensic Science
Laboratory

Dr C Wood and Janet Cowley - Chorley Forensic
Science Laboratory.

The training of Forensic Scientists

Mr P Rees - Nottingham Forensic Science Laboratory.

Discussion on the above two papers

The post graduate training of Forensic Scientists

Dr F Fish - University of Strathclyde, Division of
Forensic Science.

Command and Control in the Police Service

Dr G Turnbull - Police Scientific Development Branch

Communications Systems

Mr P P H Smith - Directorate of Telecommunications

General Discussion on the day's papers

Speaking times for the afternoon session includes 5-10

minutes for discussion

'Colloquium on
Physical Evidence'

Friday, 5 December, 1975

Chairman: Dr W Rodger

Chairmans Introductory Remarks

Best Foot Forward - Initiating foot wear casework

Mr R Hoole and D.C.I., H Mather
Chorley Forensic Science Laboratory

Some Current Methods for recording shoe marks and
making test prints

Mr R J Davies - Metropolitan Police Laboratory

Casting using Metal Spraying Equipment

Dr R N Totty - Birmingham Forensic Science
Laboratory

Some Illustrations from recent S.E.M. cases

Mr J M Dubery - Birmingham Forensic Science
Laboratory

General Discussion on the mornings papers

The Specialized use of filters in Forensic photography

Mr K E Creer - Metropolitan Police Laboratory

The Investigation of a Typewriter Defect Using High
Speed Photography

Mr M G Hall - Birmingham Forensic Science
Laboratory

The Photography of Striation and Extrusion marks on
Transparent specimens

Mr A C McWillson - Metropolitan Police Laboratory

Discussion on the Photography papers

The examination of Saw Cuts

Mr R O Andahl - Metropolitan Police Laboratory

General Discussion on Days papers

APPENDIX E

THE GEOGRAPHY OF ALDERMASTON

For UK visitors, Motorways M3 and M4 are adjacent and are shown on the map. The telephone is manned by the Director's Secretary on Tadley (07356) 3833 ext 5853. It is essential to telephone beforehand so that the necessary pass can be lodged with the police.

Trains from London (Paddington to Reading or Waterloo to Basingstoke) are advised for overseas visitors. Services are frequent and fast (30 to 50 minutes). There is also a British Railways bus link direct from London (Heathrow) Airport to Reading (50 minutes). Transport will be arranged from these railway stations which are about 10 miles away from CRE, provided that 24 hours' notice is given to the Director's Secretary.

Visitors not used to British Railways are advised that the method of opening carriage doors is first open the window and use the handle on the outside of the carriage!

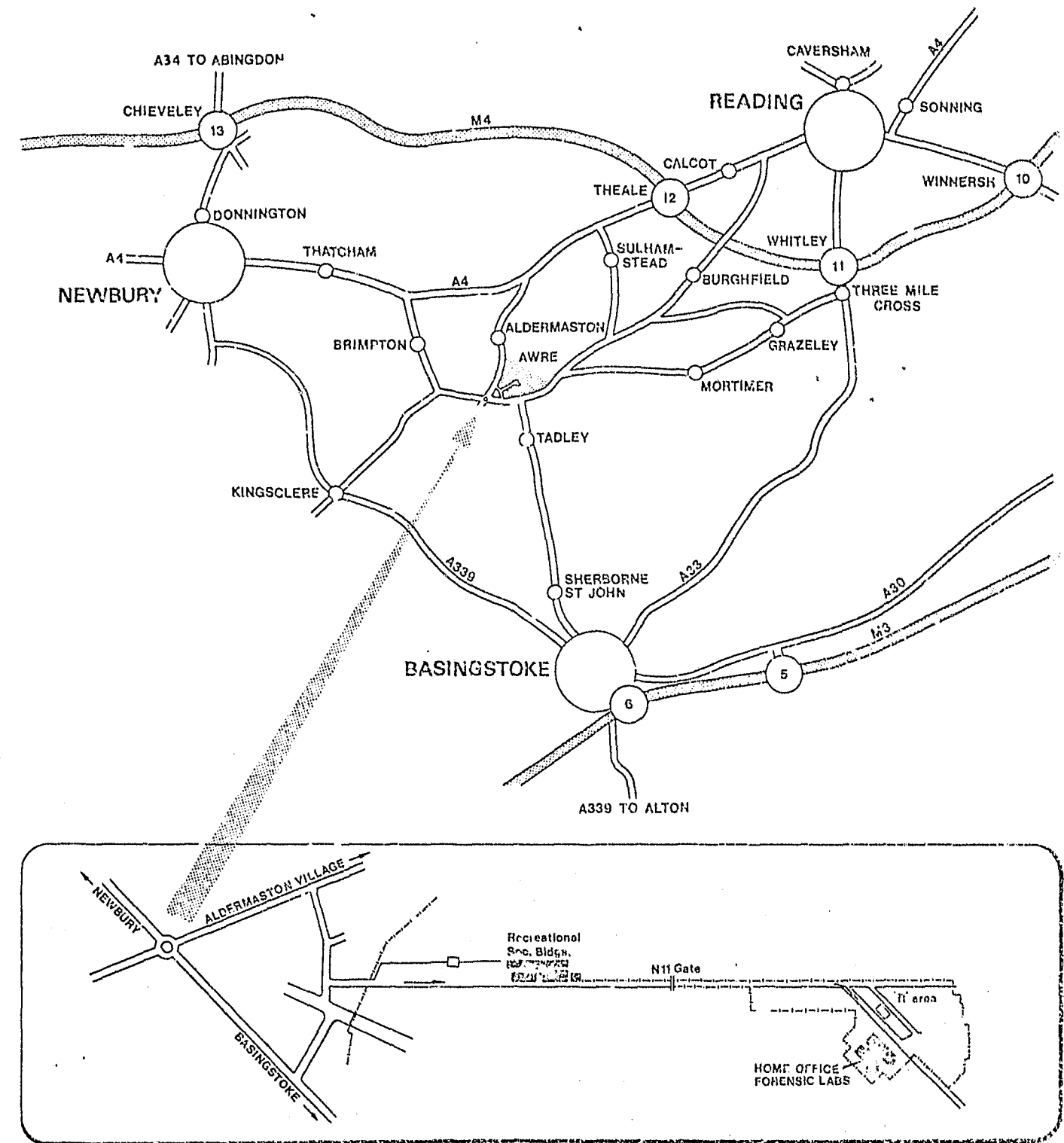


FIGURE 1. APPROACH ROADS TO AWRE AND ACCESS MAP TO HOME OFFICE LABORATORIES

END

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